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4811

Experimentally Produced Neoplasms in the Frog.*

EMIL WITSCHI.

From the Zoological Laboratory, State University of Iowa.

It has been known that the frog egg on the stage of the second polar spindle-metaphase passes into a resting period, awaiting fertilization. Under natural conditions this lasts for only a few hours, but can be delayed up to 3 days without impairing perceptibly the vigor of the egg. After a further delay of 2 days, however, the eggs are dead. In the interval the eggs gradually lose their capacity to develop into normal embryos. The abnormality of these overripe eggs becomes manifest in the following features.

1. The mechanism of control protecting normal eggs against polyspermy is impaired. As a consequence a high percentage of the overripe eggs show multiple segmentation. 2. In monosperm eggs the animal blastomeres are reduced in size, which effect seems to be due to a change in viscosity of the ovoplasm. 3. In the later development a marked tendency to produce axial duplications and supernumerary appendages is observed. These monstrosities as well as the polyspermy indicate a lack of control and coordination within the embryo and the egg. It probably is due to alterations in the cortical layer of the egg, developed during the time of overmaturing. The appearance of monstrosities during the later embryonic stages

* Aided by grants from the Elizabeth Thompson Science Fund of Harvard University.

proves that such plasmatic changes eventually persist for a long time or even become permanent. 4. In the overripe egg as well as in the cells of the embryo derived from such, one finds an overabundance of light brown pigment granules. 5. The most conspicuous pathological feature is the loss of power of differentiation in the embryonic cells and the tendency to start into neoplastic growth. Concerning this point a few details may be added.

The epidermis is thickened, consists of much enlarged cuboid cells and often by a wild growth gives rise to a characteristic epithelioma. The loss of differentiating power leads to some striking deficiencies in the head region. Often the stomodeal part of the hypophysis is incomplete or missing. Such larvae stop growing when about 25 mm. long and usually are of a light color. The formation of the lens of the eye can be omitted, even in the presence of an almost normal eye vesicle. Oftener the eye vesicles too are found undeveloped. The ear is more resistant than the eye. The central nervous system is very defective in all cases of high grade overripeness. The neural canal is missing and the neural cord consists simply of a mass of incompletely differentiated cells. Ganglia crest, spinal ganglia and the whole peripheral nervous system are even more deficient than brain and spinal cord.

In the entoderm the growth of big tumors in the intestinal wall has been noted.

The mesoderm exposes the low grade of differentiation. In the cases of high abnormality the separation into chorda and lateral mesoderm is not even attempted, while in less affected cases it is incomplete. Mesenchyme and blood cells assume unusual features. Both may appear either as round or as spindle shaped cells. In some places they are very abundant and obviously attack and infiltrate neighboring tissues. Pigment granules may be included but are not always present.

Embryos and larvae of the described type grow increasingly abnormal. They die the sooner, the more the fundamental histological structures deviate from the normal.

It was therefore essential to determine whether the abnormal tissues can be kept alive by transplantation into normal larvae and frogs. The first transplant was put under the skin of the larva right behind the ear or it was introduced into its body cavity. Most of the grafts took successfully. In their development two types can be distinguished. The first behaves like any normal graft, and after an initial growth suffers later retrogression. The second type is characterized by its infiltrating growth, which preferably spreads

through the reticular system. The wandering cells are spindle shaped or round and usually contain much pigment. In appearance it resembles the human malignant melanosarcoma, though developmentally it is not identical with this type, as it seems to be of mesodermal origin.

In a just metamorphosed frog which had carried a strongly growing implant for 22 days a metastatic nodule was found in the connective tissue between the heart and the right thyroid gland. It is made up by pigmented round and spindle shaped cells. The main bulk of the heavily infiltrated reticulum of this little frog, was transplanted, together with the attached liver and most of the intestinal tract, into the body cavity of an adult frog.

After fifty days the body of this frog began to swell and at the same time became hard. The animal soon stopped feeding and was dying when it was preserved on the sixty-second day. In the body cavity was found a large tumor which was attached to the peritoneum as well as to the mesentery and was penetrating and destroying the urinary bladder. The closer examination revealed not only a whole network of creeping ramifications through the reticulum but also numerous metastases in the liver and in the propria of the intestine.

The abnormal tissue from the overripe egg, by this double transplantation has attained an age of 92 days, while equally deficient embryos under best care do not live over 2 weeks.

4812

The Barrier Between the Blood and Cerebrospinal Fluid.

WM. MALAMUD. (Introduced by John A. Larson.)

From the Iowa Psychopathic Hospital, State University of Iowa.

In a previous communication[1] the results of investigation of this barrier by means of the Walter bromide method were reported. It was found then that the series of mental diseases investigated could be divided into 3 classes according to the distribution ratio of bromides in the blood and cerebrospinal fluid. (1) In some of these diseases the distribution ratio was about normal (about 3 times as much bromide in the blood as there was in the cerebrospinal fluid

[1] Malamud, Wm., Fuchs, D. M., and Malamud, N., *Arch. Neurol. and Psychiat.*, 1928, xx, 780.

and varying from 2.8 to 3.2). (2) In others this distribution ratio was higher. That is, less of the bromides passed into the cerebrospinal fluid. (3) In a third group the ratio was lower; that is, more bromides passed into the cerebrospinal fluid. Most of the diseases belonging to the third group were of the type where one finds affections of the smaller cerebral vessels.

In discussing the nature of this phenomenon the question was brought up as to whether this distribution of bromides could be regarded as due to a process of dialysis. The fact that there was 3 times as much of the bromides in the blood as there was in the cerebrospinal fluid apparently argued against it, for in the case of chlorides, for instance, we find a distribution of 1.0 in the blood to about 1.2 in the cerebrospinal fluid. It was suggested then that some of the bromides may be fixed in some way in the blood and rendered indiffusible. This would mean that the nature of the membrane itself, (whatever it may be) that separates the blood from the cerebrospinal fluid was not the governing factor.

Since then we have been investigating this question and some results obtained apparently argued this possibility.

1. In cases of general paralysis treated with malaria, a definite correlation was found between the distribution ratio and the clinical course of the disease. It was found that in most untreated cases there was an increased passage of bromides into the cerebrospinal fluid. Where the malarial treatment had been successful, the amount of bromides that would pass into the cerebrospinal fluid was decreased. In others it was not affected. Histopathological studies have shown that the factor influenced by malarial treatment was usually the blood vessel disease. Apparently then, the change in the distribution ratio of bromides was very definitely related to the condition of the blood vessel wall rather than the blood itself.

2. Studies on the amount of bromides that would pass into other body fluids were undertaken. Cases of pleuritic and ascitic effusions were studied. It was found in these that whereas the ratio between the blood and cerebrospinal fluid was anywhere from 2.4 to 3.0, the amounts of bromides in the blood and those in the pleuritic or ascitic fluids remained about equal. The amount of protein in these fluids did not seem to affect the ratio.

3. Blood and cerebrospinal fluid were taken from patients who had bromides administered to them. The ratio was determined and then the blood and cerebrospinal fluid were placed in a dialyzing system separated by a membrane which was permeable to bromides and impermeable to colloids. It was found that after 48 hours the

difference in the quantity of bromides in the blood and cerebrospinal fluid had gone down very appreciably. Thus, a case showing a distribution ratio of 3.15 to 1.0 had gone down to 1.21; another one of 3.37 went down to 1.27; a third of 3.52 went down to 1.2, etc. The last two experiments have just been started and are being carried on further.

4813

Studies of Action Currents in Laryngeal Nerves.

ERICH LINDEMANN. (Introduced by Lee E. Travis.)

From the State University of Iowa, Psychopathic Hospital, Iowa City, Iowa.

In order to increase the information about the neurophysiological mechanism of phonation, action currents were recorded from the inferior and superior laryngeal nerves during voice production in dogs. The whining or barking sound produced when the animal was just coming out of ether anesthesia was picked up by a condensor-microphone and amplified by a one-stage amplifier. Simultaneously with the voice the action currents appearing in the inferior and superior laryngeal nerves were picked up by platinum electrodes (in some experiments as a check-up by non-polarizable electrodes) and amplified by a 3-stage resistance coupled amplifier. The recording of both the voice and the action currents was done by means of a 3-element Westinghouse oscillograph.

The inferior laryngeal nerve was exposed just below the thyroid cartilage and the superior laryngeal nerve a few millimeters towards the entrance into the crico-thyroid membrane.

The records show that when no voice is produced the action current line is practically at rest. During voice production the action current line of the inferior laryngeal nerve shows regular oscillations having the same frequency as the voice line. The frequency of these action current oscillations changes with the pitch of the voice. In order to exclude any influence of a non-physiological source the experiment was frequently repeated with different forms of electrodes and after removing the amplifier completely from the operating room. Furthermore, if one electrode was removed from the nerve and placed upon nearby muscle tissue the regular frequencies disappeared.

In order to determine the direction in which the potential changes were traveling, the inferior laryngeal nerve was transsected and rec-

ords taken from both the central and the peripheral end. Those from the central end still presented regular oscillations of a frequency equalling the pitch of the recorded voice sound. The range of frequencies observed so far lies between 380 and 1800 oscillations per second. We, therefore, assume that the observed potential changes are travelling from the central organ to the larynx.

We do not feel justified in considering these regular action-potentials as originating in the higher centers of coordination. We studied the superior laryngeal nerve as the possible afferent branch for a proprioceptive reflex mechanism containing the inferior laryngeal nerve as efferent branch. Records taken from the superior laryngeal nerve do not show the observed regular oscillations but irregular potential changes of small amplitude and low frequency, which indicates that this nerve is largely sensory.

Transsection of the superior laryngeal nerve leads to a definite change in the action current picture recorded for the inferior laryngeal nerve. It seems that the regular oscillations described above disappear after this procedure on the side of the transsected nerve. Although this does not prove that the superior laryngeal nerve serves as the afferent branch in the reflex arc, it favors the explanation offered for the regular oscillations observed in the inferior laryngeal nerve. Preisendorfer[1] has described action current records obtained from the calf musculature while the subject is pressing his toes against a vibrating object. The usual irregular grouping of large and small oscillations was replaced by regular oscillations corresponding in frequency to the vibration of the object. He considers his picture as a series of proprioceptive reflexes. We believe that a similar mechanism is responsible for the regular oscillation observed in the laryngeal nerve. Further research will be necessary to study the suggested proprioceptive control of the action of the vocal chords.

4814

The Micro Determination of Blood Sugar.

R. B. GIBSON.

From the Laboratory of Pathological Chemistry, University Hospitals, The State University of Iowa.

A technically simple and rapid procedure for the determination of the blood sugar which gives results which are little, if at all,

[1] Preisendorfer, *Z. f. Biologie*, 1919, lxx, 505.

affected by the non-sugar reducing substances of the corpuscles and plasma is presented. The problem of non-sugar reducing substances has been approached in 2 ways—by using copper solutions which are effective on the glucose of the blood only (Folin, S. R. Benedict) and by obtaining protein-free filtrates from which the non-sugar reducing substances are removed along with the protein (Somogyi). The second procedure is obviously the more practical, and is attained in our methods by increasing the proportion of tungstate and sulfuric acid in relation to the volume of blood precipitated.

Dr. J. S. Boyd (1923), working in this laboratory, adapted the Folin-Wu method for finger-tip blood specimens to meet the increase in the number of blood-sugar determinations necessitated by insulin control of diabetics. We are now doing over 4200 blood sugar determinations yearly in the routine diabetic service alone. The procedure may be used for venous specimens as well. About 2.5 times the amounts of sodium tungstate and sulfuric acid over that in the original Folin-Wu procedure in proportion to the volume of blood taken were used as the precipitant. As completely glycolized specimens of blood give no color with Boyd's method, Shrader[1] added increasing amounts of glucose to blood after glycolysis and determined these. Calculated values for the glucose found over a range of 70 to 140 mgm. percent corresponded closely to the amounts of sugar added, with progressively increasing variation above and below these figures.

Shrader prepared a table for the glucose in mgm. percent corresponding to colorimetric readings (in mm.) over the ordinary clinical range of blood sugar values from a graph constructed from his data. The method was practical and gave results somewhat lower than could be obtained with other procedures. From some figures obtained in studying the distribution of glucose between the plasma and corpuscles in diabetic patients receiving insulin and glucose, 10 analyses gave an average corpuscle-plasma glucose ratio of 0.74, the normal ratio according to Somogyi[2] being 0.77.

However, the color developed tends to fade on standing. Substitution of S. R. Benedict's arseno-tungstate reagent with added formaldehyde[3] gives a color which is permanent and more easily matched. Both blank reagent mixtures and glycolized blood give

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[1] Gibson, R. B., Mitchell, K. Z., and Larimer, R. N., *J. Iowa State Med. Soc.*, 1925, xv, 225.

[2] Somogyi, M., *J. Biol. Chem.*, 1928, lxxviii, 117; *Arch. Int. Med.*, 1928, lxiii, 931.

[3] Benedict, S. R., *J. Biol. Chem.*, 1925, lxiv, 207.

a trace of blue color, and results for added sugar (100 mgm., to glycolized blood) are about 10 mgm. too high.

A table of glucose values corresponding to the colorimetric readings when the arseno-tungstate sugar reagent was employed was prepared by Madge L. Baltimore from determinations of known amounts of glucose added to completely glycolized blood. Her analyses were in duplicate and results obtained with 3 specimens of glycolized blood were averaged.

TABLE I.

Blood sugar values from colorimetric readings (std. at 10 mm.). Micro-adaptation for finger tip blood of the combined Folin-Wu and Benedict procedures; from sugar added to glycolized blood (M.L. Baltimore).

Mm.	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
2						400	385	372	362	349
3	337	325	316	306	297	289	281	273	266	259
4	253	247	241	236	231	226	221	217	212	208
5	204	200	196	192	189	186	182	179	176	173
6	170	167	165	162	159	157	155	152	150	147
7	145	142	140	138	135	133	131	129	126	124
8	122	120	118	116	114	112	110	109	107	105
9	104	102	100	99	98	96	95	94	92	91
10	90	89	87	86	85	84	82	81	80	79
11	78	77	75	74	73	72	71	69	68	67
12	66	65	64	63	62	61	60	59.5	59	58
13	57	56	55	54	53	52.5	52	51	50	49.5
14	49	48	47	46.5	46	45	44	43.5	43	42
15	42	41	40	39.5	39	38.5	38	37	36.5	36
16	35.5	35	34.5	34	33	32.5	32.5	32	31	31
17	30	29.5	29	29	28.5	28	27	27	26.5	26
18	25.5	25	24.5	24	24	23.5	23	23	22.5	22
19	22	21.5	21	20.5	20	20	19.5	19	19	18.5
20	18	18	17.5	17	17	17	16.5	16	16	15.5

Blood specimens from our psychopathic service probably more closely approximate normal venous blood values. For evaluation of the blood sugar method, 107 specimens of blood from these patients for the 2nd half of the year 1929 have shown an average of 91 mgm. percent with extremes of 66 to 122 mgm. A second routine series beginning January 1, 1930, of 25 specimens shows an average of 85 mgm. percent with extremes of 72 to 104 mgm. percent. The distribution of glucose in the venous blood during a sugar tolerance test on a mild diabetic gave a corpuscle-plasma ratio of 0.76 as an average for 6 blood specimens taken. The corpuscle glucose content was calculated from analyses of whole blood, plasma, and the hematokrit value.

Draw 0.2 cc. of blood from a finger tip puncture (we use a pointed interchangeable surgical knife blade) into a serological or special pipette, graduated to the tip, as in drawing blood for the

Sahli hemoglobin determination. Discharge the blood into a centrifuge tube containing 4.3 cc. of 1.25% sodium tungstate solution and 0.5 cc. of 2% (volume) sulfuric acid. Centrifuge after standing 15 minutes; there will be sufficient supernatant fluid for duplicate determinations. Take 2 cc. of the supernatant fluid in a Folin-Wu sugar tube graduated at 10 and 25 cc. and 2 cc. of 0.01% glucose standard solution in a second tube; add 2 cc. of the alkaline copper-tartrate solution to each tube, and heat for exactly 6 minutes in the boiling water bath. Cool, and then add 2 cc. of sugar reagent to each tube and mix by inclining the tubes and tapping the bulbs of the tubes sharply against the palm of the hand until gas ceases to form. Dilute the blood sugar tube to the 10 cc. mark and the standard tube to 25 cc., mix, and read in the colorimeter with the standard at 10 mm. Take the result from tabulated values for the ordinary range of readings. Blood specimens sent to the laboratory may be determined by the above procedure; 0.05 gm. of sodium fluoride per 5 cc. of blood is preferred as a preservative and anticoagulant.

For bloods with blood sugar values over 300 mgm., the blood tube may be diluted to the 25 cc. mark and the calculated result multiplied by 2.5. For hypoglycemic bloods a double strength alkaline copper solution is used; for 2 cc. of the supernatant fluid, add 1 cc. of the double strength copper solution and 1 cc. of a 0.004% glucose solution and subtract 50 mgm. from the blood sugar figure obtained.

Solutions. 1. Sodium tungstate, 1.25% solution.

2. 2% sulfuric acid by volume (approximately 2/3 normal).

3. Alkaline copper-tartrate solution: dissolve 16 gm. of anhydrous sodium carbonate in 160 cc. of water in a 400 cc. beaker, add 3 gm. of tartaric acid, and when dissolved add 1.8 gm. of crystalline copper sulfate (grind in a mortar after weighing); mix and make up to 400 cc. (filter if necessary). A double strength copper solution, twice the above ingredients made up to 400 cc., should be kept on hand.

4. Standard sugar solution (stock): 1 gm. of glucose to 100 cc.; add NaF as a preservative and keep in the ice-box. Dilute 1 cc. of the stock solution to 100 cc. to give a 0.01% solution. Dilute 1 cc. to 250 cc. for a 0.004% solution.

5. Arseno-phosphotungstic acid reagent: 100 gm. pure sodium tungstate in a liter flask with 600 cc. water and dissolve. Add 50 gm. of pure arsenic pentoxide, 25 cc. of 85% phosphoric acid, 20 cc. of conc. hydrochloric acid. Boil 20 minutes. Cool and dilute to 1000 cc. Add 5 cc. of 40% formaldehyde solution for each 100 cc. of the reagent.

Missouri Section.

Washington University School of Medicine, February 12, 1930.

4815

Observations on the Formation of Wheals. II. Comparison of Wheals Induced by Allergens and by Histamin.

H. L. ALEXANDER, J. O. HARTER AND F. S. MC CONNELL.

From the Department of Medicine, Washington University Medical School, and The Barnard Free Skin and Cancer Hospital, St. Louis, Missouri.

The lesion of allergy (used in the sense of atopy) is essentially a localized edema, whether or not smooth muscle spasm occurs also. The edema of the bronchi in asthma, the swelling of the nasal mucosa in hay fever, the wheal of urticaria and the positive skin reaction in an allergic individual all show the same histological picture. With the hope of throwing some light on tissue participation in allergy, a study of wheal formation was undertaken.

In a previous publication,[1] it was shown that when intradermal tests were done with an offending allergen at different sites in an allergic individual, the resulting wheal varied greatly in size. This occurred despite the fact that the dose of the injected allergen was constant (0.02 ccm.). Wheals laid down on the back and abdomen were uniformly much larger than those on the extremities, and those on the upper outer thigh were intermediate in size.

The wheal of a positive skin test results presumably from a reaction between the injected allergen, and antibody in the skin tissues, with the consequent formation of some substance having a resemblance to histamin. This, in turn, acts directly upon the walls of the surrounding capillaries. These dilate and become permeable to their contained plasma which comes out into the tissues and forms the wheal.

In order to analyze which of these factors may be responsible for the discrepancy in the size of wheals in different sites on the skin in response to a constant amount of allergen, the following experiment was performed:

[1] Alexander, H. L., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 800.

Normal individuals were injected intradermally with 0.02 ccm. of a 1 to 2500 dilution of histamin phosphate in the interscapular region, the flexor surface of the left forearm and in the upper outer aspect of the right thigh. Two wheals were laid down on each site. Unless each pair of wheals were approximately of the same size, the subject was not used. A blue-barrel tuberculin syringe with a special attachment calibrated in 0.01 ccm. was used. The wheals were allowed to increase in size for 15 minutes and were then traced in ink.

In the 25 subjects tested, it was found that the largest wheals occurred on the back, the smallest on the forearm and those on the thigh were intermediate in size. Since this result is similar to that found with allergens, the experiment was repeated in allergic subjects who then received both histamin and allergens at the above 3 sites. If the dose of histamin happened to correspond to the dilution of allergens used, wheals of similar size and character were obtained at each site.

The outlines of wheals obtained at the 3 sites in 18 allergic subjects with allergens and in 14 normals with histamin were measured with a planimeter. The average sizes of these wheals were as follows:

	Back	Forearm	Thigh
Allergen	2.04 sq. cm.	1.14 sq. cm.	1.55 sq. cm.
Histamin	2.00 " "	1.35 " "	1.78 " "

In a recent publication by Gröbel,[2] similar results were obtained by producing wheals with morphine in normal subjects. He found a larger response on the trunk than on the extremities. Apparently, the cause of this discrepancy in allergic individuals is the same as that in normal subjects. Consequently, one may rule out immunological factors, such as irregular antibody distribution and failure of allergen and antibody to form a capillary dilating substance.

A final possibility, according to present conception, is a variability in response of skin capillaries at different sites. This was examined by experiments in dogs. In these animals there is a similar discrepancy in size of wheals to histamin, the skin of the thigh giving larger reactions than that of the leg to a 1 to 5000 dilution. This dilution was injected into the femoral artery and a diffuse redness appeared in the skin of the entire extremity, showing an apparently uniform capillary reaction. Although such an experiment is open to criticism, it at least throws doubt on the highly conjectural supposition that there is a great difference in response to histamin inherent in capillaries of the skin of the trunk compared to that of

[2] Gröbel, F., *Z. f. d. ges. Exp. Med.*, 1929, lxv, 352.

the extremities. Some other factor seems necessary to account for this discrepancy of skin response. This is dealt with in the paper following.

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Observations on the Formation of Wheals. III. The Participation of an Unidentified Tissue Substance.

H. L. ALEXANDER, W. K. WEAVER AND F. S. MC CONNELL.

From the Department of Medicine, Washington University Medical School, and The Barnard Free Skin and Cancer Hospital, St. Louis, Missouri.

In previous publications[1, 2] it was shown that if in an allergic individual, intradermal tests be done with an offending allergen, the resulting wheal will vary in size depending upon the site of the skin injected. Larger responses occur on the trunk than on the extremities. The same discrepancy occurs in normal subjects with histamin, morphine and atropin, all of which produce wheals in normal skin. The cause of this variability in response is apparently not due to immunological factors and probably not to inherent lack of response in the capillaries. Consequently, there seems to be some other participating factor and this was sought in the skin tissue itself.

Advantage was taken of the fact that a histamin wheal in a dog's skin is similar in time of formation, shape and histological picture as that in human skin. Moreover, the same discrepancy in size of wheal formation at various sites, to an intradermal injection of a constant amount of a given strength of histamin occurs in a dog just as it does in humans.

Dogs were anesthetized with amytal and a portion of the shaved skin of the abdomen dissected off. This was washed in 0.85% sodium chloride solution until free from blood. The subcutaneous fat was dissected off and the skin cut into fine pieces. Ten grams of washed chopped skin were placed in 90 cc. of 0.85% saline solution to which sufficient histamin phosphate had been added to make a final dilution of this drug 1 to 10,000. The mixture was thoroughly shaken at intervals for 2 hours and filtered. For a control, proportionate amounts of skin and saline solutions without histamin were prepared in the same way. These mixtures were tested

[1] Alexander, H. L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 800.

[2] Alexander, H. L., Harter, J. O., and McConnell, F. S., *Ibid.*, 1930, xxvii, 484.

in a second dog under amytal anesthesia. Three intradermal injections of 0.02 cc. each into the shaved skin of the abdomen were made as follows: (1) Histamin phosphate 1 to 10,000 dilution, (2) Saline extract of skin (1 part in 10) containing histamin phosphate in 1 to 10,000 dilution, (3) Saline extract of skin without histamin.

Planimeter measurements were made of both the initial and the enlarged wheal. The difference between the 2 measurements represents the increase in size in sq. cm. This is recorded in Table I.

TABLE I.

H	*S*	*H*	*S*
0.2	0.8	0.5	1.0
0.2	0.7	0.2	0.6
0.3	0.9	0.3	0.9
0.4	0.9	0.2	0.8
0.3	0.8		

H = Histamine 10,000. S = Skin Extract + Histamine 10,000.
Each pair of figures represents one experiment—0.02 cc. of fluid injected. Figures record in sq. cm. increase of size of wheals after 15 minutes.

Several hundred such wheals were measured. It is apparent that there is something in skin tissue which augments histamin inasmuch as wheals induced with histamin and skin extract were uniformly larger than those made by histamin alone.

That the tissue factor is not histamin is evident from the skin extract control and also because the same increase is obtained with atropin and codein. This tissue factor is contained in organs other than skin, notably lung and liver.

Although further investigations are being carried on, it appears possible that wheal formation is dependent on this tissue substance. This may explain the discrepancy in the size of wheals induced in various parts of the skin by assuming that more of the substance is contained in the skin of the trunk than in that of the extremities. Further study of this phenomenon may throw some light on tissue response in allergy in which the wheal is the essential lesion. Chemical studies on the identification of this tissue factor are being conducted.

4817

The Shortening of the Coagulation Time of the Blood by Irradiated Ergosterol.

W. C. CORSON, G. F. IRWIN AND R. A. PHILLIPS.
(Introduced by Evarts A. Graham.)

From the Department of Surgery, Washington University School of Medicine and Barnes Hospital, St. Louis, Missouri.

This is a report of further studies on the effect of irradiated ergosterol on the mammalian thrombocyte counts begun by Phillips and Robertson[1] in this laboratory. They found that the thrombocyte count was markedly increased by the administration of irradiated ergosterol. In view of these findings and the results obtained by F. D. Gunn,[2] who produced a distinct rise in the number of thrombocytes by exposing young rabbits to radiations from the mercury vapor lamp, it was reasonable to expect a corresponding decrease in the clotting time.

In our experiments the Sooy and Laurens[3] method was used for counting thrombocytes as described by Phillips and Robertson.[1] For coagulation tests a modification of Boggs'[4] method was used, of which a brief description is here given: A small metal box with a glass bottom was covered by a tightly fitting glass cone. A round drop of blood was placed upon the small end of the cone and quickly inverted into the box. The instrument was placed on the stage of the microscope and the edge of the drop examined with the 16 mm. objective. A small stream of air was then directed on the edge of the drop at 15 second intervals. Instead of blowing directly into the chamber as described by Boggs, the air was passed through a wash bottle that was immersed in a water bath to keep the temperature and the humidity of the air constant. Coagulation was assumed to be completed when the corpuscles moved en masse, and sprang back to their original position.

White rats of various weights and unknown age were selected at random. Ten such rats were fed 3 minims of viosterol* daily with a medicine dropper for 9 days. Four rats were fed 3 minims for 2

[1] Phillips, R. A., and Robertson, D. F., *Trans. Soc. Exp. Biol. and Med.*, 1929, xxvi, 639.

[2] Gunn, F. D., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 120.

[3] Sooy, J. W., and Laurens, H., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii.

[4] Boggs, T. R., *Internal. Clin. Phila.*, 1908, i, 31-39.

* A preparation of irradiated ergosterol furnished to us through the courtesy of Mead, Johnson and Company.

days. Four rats were fed 3 minims for one day. Adequate control rats were used with each series, and these failed to show any noteworthy change. A total number of 18 animals were followed for 12 days and on each animal thrombocyte counts and determinations of coagulation time were made daily.

The following results were obtained: The normal coagulation time varied between 1 minute and 45 seconds and 2 minutes and 45 seconds, with an average of 2 minutes and 10 seconds. The normal thrombocyte and red blood cell counts for the white rats agreed with those of Cramer, Drew and Mottram,[5] *viz.*, average red blood corpuscles were 8,500,000 to 10,000,000 per cm. and the average thrombocyte count from 500,000 to 700,000.

Each animal receiving viosterol showed a marked decrease in the coagulation time which occurred simultaneously with the marked increase in the thrombocyte count. The results of Phillips and Robertson[1] were confirmed in that the thrombocyte count was doubled in 48 hours. The highest counts recorded, which were as high as 3,000,000 from a normal count of 600,000, were obtained on the fifth through the seventh day, after the initial dosage. The lowest coagulation time (15 seconds to 30 seconds) occurred also on the fifth through the seventh day. This is readily observed in Table I, which represents 5 typical animals.

TABLE I.

Animals	No. of Doses Administered	Normal Thrombocyte Count	Highest Thrombocyte Count	Normal Clotting Time	Lowest Clotting Time	Day of Occurrence
Rat I	Continuous	612,500	2,250,000	2:00 min.	45 sec.	5th
Rat II	Continuous	664,500	3,132,000	1:45 "	15 "	5th
Rat III	2	712,000	2,345,000	2:15 "	30 "	7th
Rat IV	1	661,000	2,466,000	1:45 "	45 "	5th
Control	0	560,000	654,000	1:45 "	1:45 min.	.....

The series of rats which received only one or 2 doses showed approximately the same results as those which received continued doses. The continued dosage tended to produce a hypervitaminosis, as shown by a diarrhea, dullness, sluggishness, emaciation and a loss of hair, whereas, the smaller number of doses gave no evidence of hypervitaminosis.

It is believed that the series of animals presented was large enough to prove definitely that the coagulation time of the blood can be shortened, at least in normal rats, by feeding irradiated ergosterol.

[5] Cramer, Drew and Mottram, *Proc. Royal Soc. London*, 1922, xciii, 449.

We hope to demonstrate by further experiments that the coagulation time which is prolonged in obstructive jaundice can be shortened sufficiently to reduce the operative risk.

4818

Comparison of Effects of Various Preparations of Anterior Pituitary Gland on Thyroid of Guinea Pig.

LEO LOEB AND R. B. BASSETT.

From the Department of Pathology, Washington University School of Medicine, St. Louis, Missouri.

In a former communication,[1] we reported that the thyroid gland of guinea pigs which received injections of acid or alkaline extract of the anterior pituitary gland within a short time showed such a remarkable hypertrophy that it closely resembled the thyroid seen in pronounced cases of Graves disease. These findings were in marked contrast to the fact that the feeding of Armour's tablets of anterior pituitary prevented or greatly inhibited compensatory hypertrophy in thyroidectomized guinea pigs.

We now report further observations on the effect of the anterior pituitary gland.

Preparation of Extracts. The anterior pituitary of cattle was freed completely from other parts of the hypophysis, then dried and powdered. Five grams of the dried powder were extracted with 100 cc. of 0.5% acetic acid or with 100 cc. of 0.1% sodium hydroxide for a period of 24 hours, in ice chest. The fluid was separated from the residue by filtration, neutralized to pH 7.8 (Phenol Red), refiltered to remove a protein precipitate which falls out at the isoelectric point. The filtrate was then passed through a Seitz bacterial filter to render it sterile.

Experimental. Silberberg,[2] in the course of experiments carried out in this laboratory observed that there was a distinct hypertrophy in the thyroid gland of the guinea pig as early as one day after the injection of 1 cc. of acid extract of the anterior pituitary. Following this, we made a comparative study of the action of acid and alkaline extracts on the thyroid gland and also on the sex organs. One group of animals was injected daily with 1 cc. acid extract, and another

[1] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 860.

[2] Silberberg, Martin, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 166.

with 1 cc. of alkaline extract. On each day, beginning with the second day of the experiment and ending with the seventh, we removed the thyroid gland from one guinea pig of each set. On the whole, we observed that the curves representing hypertrophic changes were the same for both extracts. A noticeable hypertrophy was seen even after one injection. The hypertrophy increased with the number of injections until the fifth or sixth day when the maximum was reached. But at the end of the second or third day the maximum of mitotic proliferation was reached, at which time the number of mitoses was exceedingly great. After this, the mitoses decreased while the solution of the colloid and the hypertrophy of the acinus cells progressed. Thus hypertrophy takes place with a surprising rapidity and the maximum increase in mitotic activity is reached before the stage of maximum hypertrophy and absorption of colloid. There is no marked difference in these respects between the action of acid and alkaline extracts.

In one experiment, an intravenous injection of 1 cc. of acid extract was given and one day later the thyroid removed for microscopic examination. A study of the gland showed the presence of early hypertrophy.

Other experiments were performed in which several cc. of extract were injected daily to compare the intensity of hypertrophy with that caused by 1 cc. daily. Contrary to expectation, the former procedure did not cause any greater hypertrophy than the latter.

In contrast to the thyroid of the guinea pig, which responds with remarkable regularity to the effect of anterior pituitary extracts, the rat thyroid does not show distinct hypertrophic changes after daily injections of 0.5 to 1 cc. of acid extract. At the most, only a trace of hypertrophy was demonstrable. We may conclude then, that the thyroid of the guinea pig is much more responsive to the injection of the extracts than that of the rat.

After determining the principal effects of acid and alkaline extracts on the thyroid of the guinea pig, we studied the effect of daily subcutaneous inoculations of a whole anterior pituitary gland taken from another guinea pig and also, of small pieces taken from cattle gland. In the greater number of the experiments, 6 to 10 inoculations were made on successive days, in the remaining, the number of inoculations was less. It was found that the subcutaneous inoculation of cattle gland was much more effective in causing hypertrophic changes in the thyroid than was the guinea pig gland. This may have resulted from the use of a comparatively greater amount of gland substance in the former case. This mode of ap-

plication was, however, less effective than the intraperitoneal injection of either the acid or alkaline extract.

We have also investigated the effect of these substances on the sex organs of the guinea pig. This phase of our studies is not yet complete and we shall report on it more fully at a later date. But we may state that the effects on the thyroid gland and those on the sex organs do not parallel each other. For example, inoculation of anterior pituitary gland of the guinea pig is very effective in causing ovulation, hypertrophy of the uterus and the opening of the vagina, whereas, it has only a relatively slight effect in causing hypertrophy of the thyroid. Inoculation of anterior pituitary of rabbit is likewise effective on the sex organs of the guinea pig. On the other hand, inoculation of anterior pituitary of cattle apparently does not produce changes in the sex organs as observed above, yet it is effective in causing hypertrophy of the thyroid. Acid or alkaline extracts, as prepared in our laboratory, do not cause ovulation or opening of the vagina in the guinea pig, but do lead to the production of structures which correspond to the so-called interstitial gland in the ovary of the rabbit. Such marked hypertrophy of the theca constituents of atretic follicles may lead even to the production of structures which somewhat resemble small corpora lutea. However, these structures contain degenerating ova.

These findings suggest that there may possibly be several constituents in the anterior pituitary gland, each of which acts in a specific manner on the thyroid gland and on the sex organs.

4819

Interaction Between Substances in Tissue Extracts and Blood Sera. Effect of Mixtures of these Substances on Coagulation of Blood.

E. L. BURNS, F. H. SCHARLES AND L. F. AITKEN. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Loeb[1, 2] and subsequently Hewlett,[2] Muraschew,[2] and Nolf,[2] have shown that tissue coagulins (thrombokinase of Morawitz, tissue fibrinogen of Wooldridge and Mills, thromboplastic substances of

[1] Loeb, Leo, *Montreal Med. J.*, 1903, xxxii, 507; *Virchow's Archiv.*, 1904, clxxvi, 10.

[2] Loeb, Leo. See review of older literature in *Biochem. Centralblatt*, 1907, vi, 829.

Schmidt and Howell, cytozym of Fuld, Spiro, and Bordet) are specific in different species of animals, the tissue coagulin of one species being relatively more efficient in causing coagulation of plasma from that species than from any other. Loeb,[3] and subsequently Loeb, Fleisher and Tuttle,[4] also found that when tissue extracts and blood sera are mixed together and incubated for varying periods of time, coagulation of plasma, added at the end of incubation, was delayed. In general, the longer the period of incubation the greater was the degree of inhibition of coagulation. Some evidence also pointed to a specific interaction between extracts and sera from homologous species and there was an indication that both accelerating and inhibiting effects were due to the interaction of substances specifically adapted to each other. The following experiments were carried out in an attempt to confirm and extend these observations.

Method. Constant amounts of kidney extracts and blood sera were incubated at 34° C. for periods varying from 0 to 80 minutes. At the end of these periods 1.0 cc. of heparized blood plasma was added and the time necessary for coagulation noted. Controls were made by substituting 0.9% NaCl solution for blood serum. Dog, goose, and chicken heparized plasma were used, and serum and kidney extracts were taken from the human, dog, rabbit, ox, sheep, goose, and chicken.

Results. Dog Serum with Various Kidney Extracts. Dog serum in combination with various kidney extracts produced inhibition of coagulation which became more pronounced with longer periods of incubation. In some cases an initial acceleration was noted. The degree of inhibition was always greater when dog kidney extract and dog serum were incubated together.

Human Serum with Various Kidney Extracts. Inhibitory effects were noticed when human serum was combined with human kidney extract or dog kidney extract. In many cases homologous combinations gave more inhibition than heterologous combinations, but in some experiments the reverse was noted, probably in consequence of the larger amount of inhibiting substance present in dog kidney extract. In such cases a relative specificity could be demonstrated.

Sheep Serum with Various Kidney Extracts. A specific acceleration of coagulation occurred when sheep serum and sheep kidney

[3] Loeb, Leo, *Hofmeister's Beitrage,* 1904, v, 534; 1907, ix, 185.

[4] Loeb, Leo, Fleisher, M. S., and Tuttle, L., *J. Biol. Chem.,* 1922, i, 1, 461 and 485.

extract were combined. With other extracts a mild inhibition was noted.

Ox Serum with Various Kidney Extracts. Acceleration of coagulation was also noted with combinations of ox serum and ox kidney extract, while with heterologous extracts, the absence of specific interaction between accelerating substances allowed mild inhibition to occur.

Rabbit Serum with Various Kidney Extracts. Varying degrees of inhibition without uniformity were noted when rabbit serum was combined with various kidney extracts.

Goose Serum with Various Extracts. Goose serum when incubated with goose kidney extract gave very marked inhibition, while combinations with other extracts produced only mild degrees of inhibition. These results obtained with both dog and goose plasma.

Chicken Serum with Various Kidney Extracts. Little change was noted when chicken serum was incubated with chicken kidney extract, but usually a mild inhibition occurred after longer incubation periods. Somewhat more marked inhibition was noted with heterologous combinations. In contrast to the experiments with goose serum and extract, where the action of these combinations was compared on goose as well as dog plasma, in the case of chicken serum extract, dog plasma alone was used. This, perhaps, may explain the indefinite results obtained with these latter combinations.

Discussion. These experiments are in agreement with the conclusions of Loeb that when blood serum and tissue extracts are incubated together, there are two substances which affect the coagulation of the plasma. One of these factors causes an acceleration beyond that which occurs when tissue extracts alone are added to the plasma and the other causes an inhibition of coagulation. The accelerating factors are evidenced first by the initial acceleration noted after short incubation periods, and by the pronounced acceleration observed in combinations of ox serum with ox extract, and sheep serum with sheep extract. The inhibitory substances manifest themselves in combinations of dog serum with various extracts especially, but also with human, rabbit, and goose serum.

Not only do these experiments indicate the presence of accelerating and inhibiting substances, but they also point to a specific adaptation between these factors in sera and extracts from homologous species. Thus the most marked inhibition is observed when dog serum is combined with dog kidney extract rather than with any other extracts. Human serum is always relatively or absolutely more effective in causing inhibition when combined with human

kidney extract than with dog kidney extract. Again, goose serum is much more effective in causing inhibition when combined with goose kidney extract. On the other hand, in those sera and extracts which tend to cause acceleration of coagulation, most marked accelerations are always noted when homologous combinations are used. Thus the greatest acceleration is noted when ox serum is combined with ox kidney extract or when sheep serum is combined with sheep kidney extract.

From these data it appears that not only are accelerating and inhibiting substances developed in combinations of sera and extracts, but that the latter are specifically adapted to each other so as to be most effective when homologous factors are combined.

4820

Oral Administration of Anterior Pituitary Tablets and Our Laboratory Preparations on Compensatory Hypertrophy of Thyroid Gland.

LEO LOEB AND WALTER J. SIEBERT.

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

In earlier investigations Loeb,[1] and Loeb and Kaplan[2] have shown that the compensatory hypertrophy of the thyroid gland of guinea pigs which takes place after extirpation of a great part of this organ, is very much diminished or entirely prevented if, following the extirpation, daily a tablet of Armour's anterior pituitary substance is fed to guinea pigs. In our first publication, we considered whether the effect observed by us was due to the anterior pituitary preparations as such or to an admixture. Analysis of the action of iodine preparations on the compensatory hypertrophy of the thyroid gland allowed us to exclude the addition of this substance as the cause of the prevention of compensatory hypertrophy. Furthermore, H. A. McCordock[3] showed in this laboratory that Armour's tablets prevent also the marked increase in mitoses in the thyroid otherwise produced by administration of KI to guinea pigs. We had planned several years ago to compare with the effect of Armour's preparation, the effect of oral administration of anterior pituitary of cattle prepared by

[1] Loeb, Leo, *J. Med. Res.*, 1920, xl, 481; *Am. J. Path.*, 1929, v, 71.

[2] Loeb, Leo, and Kaplan, E. E., *J. Med. Res.*, 1924, xliv, 557.

[3] McCordock, H. A., *Am. J. Path.*, 1929, v, 171.

ourselves from fresh anterior pituitary obtained from the slaughter house. Only within the last year was it possible for us to carry out these plans. We dried the anterior pituitary of cattle after it had been cut into small pieces, and powdered it. Pills were then made from the powdered substance, each pill containing the same amount of anterior pituitary substance as one tablet of Armour & Co. We used for our experiments 54 guinea pigs. From each animal the thyroid lobe of one side and either one-half or two-thirds of the other side was removed. One-third of the animals was fed daily with tablets of the Armour preparation, and a second third was fed with pills prepared in our laboratory, while the last third did not receive anterior substance; it served as a control set. The remaining parts of the thyroid gland were removed for microscopic examination at periods varying between 20 and 30 days. Our results can be briefly stated as follows:

In every case the acinar epithelium of the thyroid of the guinea pigs fed with Armour's preparation was, on the whole, low and the colloid hard; the acini themselves were relatively small. Peripheral vacuolization in the colloid was usually lacking and mitoses were not seen. However, in some cases, a small area of the periphery of the remaining part of the gland showed some increase in size of the epithelium and a loss of colloid. In all probability we have to deal here with a localized stimulating effect of the cut or of the nearness of the ligature; such a change was not found throughout the gland, as is the case in controls when we have to deal with real compensatory hypertrophy.

The remaining parts of the thyroid of guinea pigs fed with anterior pituitary gland prepared in our laboratory behaved quite differently. This preparation did not prevent compensatory hypertrophy. It is probable that it diminished it slightly, but there occurred some variations in this respect. For instance in one case the control animal had lost considerable weight and died at the end of the experiment. The thyroid tissue of the guinea pig fed with our preparation of anterior pituitary showed accordingly more hypertrophy than the control. However, we can state that our own preparation of anterior pituitary did not prevent compensatory hypertrophy; it was much less effective than Armour's preparation. This was already noticeable at the time of the removal of the remaining part of the gland for purposes of microscopic examination; the guinea pigs fed with Armour tablets showed the smallest remnants of the thyroid. On the average, all 3 classes of guinea pigs gained in weight during the course of the experiment; but those fed

with Armour's preparation gained much less than the others. However, there was some variation in this respect between individual animals and the difference in the weight curves cannot account for the lack of compensatory hypertrophy in the group fed with Armour's tablets.

We have no reason for assuming that the cause of this difference is due to the admixture of another substance to the anterior pituitary. Dr. F. Fenger was kind enough to give us a detailed description of their mode of preparing the Armour anterior pituitary tablets. There are some differences in their and our own technique, and it is possible that this accounts for the differences in the effects obtained with the 2 preparations. In experiments which we have already begun, we shall attempt to separate the 2 substances responsible for the 2 opposite effects on the thyroid gland of the guinea pig which we found in certain preparations of anterior pituitary gland.

4821

Effect of Anoxemia, Carbon Dioxide and Lactic Acid on the Autonomic Fibers of Somatic and Visceral Nerves.

PETER HEINBECKER. (Introduced by Evarts A. Graham.)

From the Department of Surgery, Washington University School of Medicine, St. Louis, Missouri.

In a previous paper,[1] the effects of anoxemia, CO_2 and lactic acid on certain fibers of somatic nerves were described. Further investigations by the author[2] and by Heinbecker and Bishop[3] have identified in autonomic nerves 2 other components of potential which possess properties different from those previously described by Gasser and Erlanger.[4] Similar action potentials traced by Erlanger and Gasser[5] from sympathetic rami into somatic nerves and thoroughly studied there by them apparently arise from similar fibers. We have found their potentials to have the same properties there as in autonomic nerves. These fibers have a higher threshold, a slower conduction

[1] Heinbecker, Peter, *Am. J. Physiol.*, 1929, lxxxix, 58.

[2] Heinbecker, Peter, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 349.

[3] Heinbecker, P., and Bishop, George H., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 645.

[4] Gasser, H. S., and Erlanger, Joseph, *Am. J. Physiol.*, 1927, lxxx, 522.

[5] Erlanger, Joseph, and Gasser, H. S., *Am. J. Physiol.*, 1929, in press.

rate, a longer absolutely and relatively refractory period, a longer chronaxie, and a potential at the stimulus of longer duration.

With the use of higher amplification (200 mm. per mv.) than employed in the previous work on CO_2, the compound conducted action potential of sciatic nerves of the frog, turtle, cat, dog and the monkey give rise to the same 4 components of potentials, named A, B_1, B_2 and C as are found in autonomic nerves. Taking the frog's sciatic as a type, the A wave arises from thickly myelinated fibers (somatic motor and sensory) as previously shown by Erlanger.[6] The B_1 wave presumed to correspond to the Delta wave described by Erlanger and Gasser but now with higher amplification no longer recognized by them as a separate entity is considered by the author, both because of its conduction rate and its other properties, to correspond to the B_1 wave described by the author[7] and by Bishop and Heinbecker.[4] In autonomic nerves it has been shown to arise from larger relatively thinly myelinated visceral afferent fibers. The results previously presented consequently cover the A and the B_1 potential waves of the new nomenclature. The materials used have been chiefly the sciatic nerves of bull frogs and autonomic nerves of the turtle.

Under anoxemia and lactic acid (N/1) the threshold of the B_2 and C fibers is finally raised. There is usually a definite preliminary lowering under anoxemia. With pure CO_2 the threshold of the B_2 fibers is little altered or moderately raised or lowered. That of the C fibers is usually quite definitely lowered. The conduction rates always are ultimately diminished. The absolutely refractory periods are increased. These findings apply to all 3 agents. Under anoxemia and lactic acid (N/1) the conducted action potential increases in temporal dispersion and its amplitude is decreased, ultimately to extinction, the B_2 and the C before the A, the C before the B_2 component. Under CO_2 the B_2 potential wave is moderately depressed, the C potential little altered or increased. Recovery with oxygen and washing with Ringer's is rapid but less so than with the A component and accompanied by a definite increase in total area above normal. Conduction rate and absolutely refractory period in good preparations return to normal. Threshold usually is lowered below the normal with recovery. Especially in the early stages of recovery there frequently occurs in these processes as was found previously for the A and B_1 processes, a dissociation of ordinarily correlated properties.

[6] Erlanger, Joseph, *Am. J. Physiol.*, 1927, lxxxii, 644.

[7] Heinbecker, Peter, *Am. J. Physiol.*, 1930, in press.

It has been observed that the B_2 and C potential waves are diphasic even when the nerve is killed between leads in a manner to render the A process apparently monophasic. When the nerve is killed only at the distal lead leaving the B_2 and C record almost completely

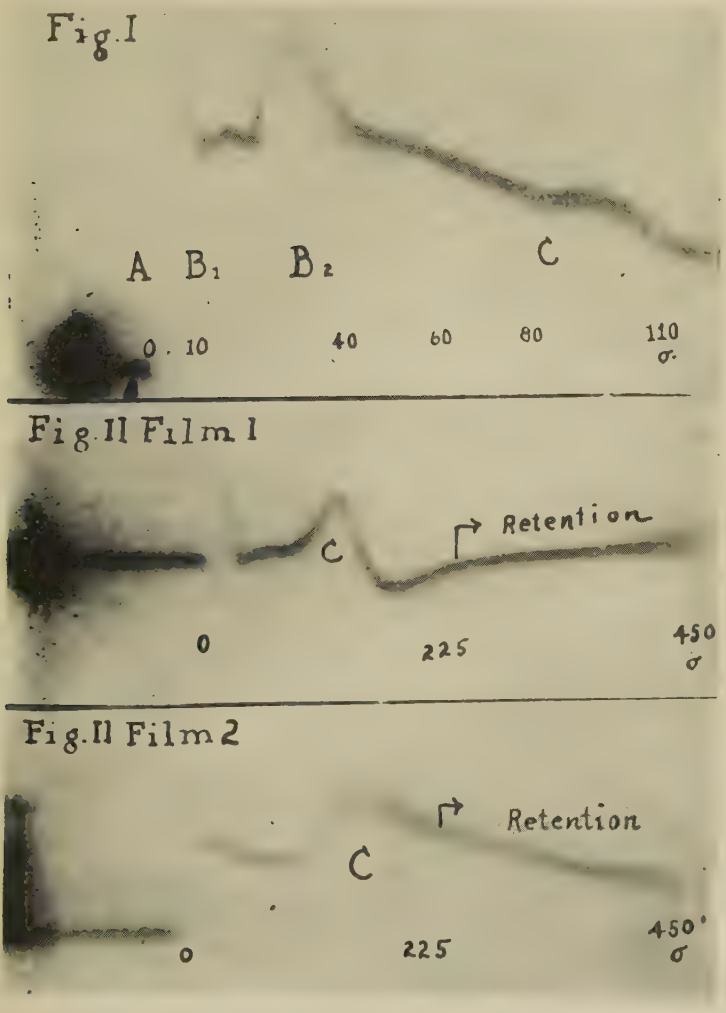

FIG. 1. Conducted action potential bullfrog sciatic after 20 minutes in pure CO_2. Note 4 potential components. Rates are B_1—3.5, B_2—1.9, C—0.5 m.p.s. as contrasted with the normal B_1—9.5, B_2—3.6, C—0.6 m.p.s. A rates not measured.

FIG. 2. Film 1. Conducted action potential normal turtle cervical sympathetic trunk showing particularly the C wave and the retained portion of the action potential. Record is quite diphasic.

FIG. II. Film 2. Conducted action potential same nerve after 3 minutes in oxygen on recovery from CO_2. Record is *apparently* monophasic because recovery at the anode is still slight. Note marked increase in height of retention.

diphasic (2 equal phases), anoxemia, carbon dioxide and lactic acid depress the second phase materially before the first phase is much altered indicating an increased susceptibility to these agents near the injured point. In partly diaphasic records retention of the second phase presumably accounts for the late positive "after-effects" reported by Amberson and Downing[9] and others. In fact, by consideration of the possibilities of differential depression or augmentation at the 2 leads, one suffering depression due to killing near the distal electrode, it is possible to account for the variations of the potential form, reported by recent authors, under anoxemia and CO_2. During depression with anoxemia and carbon dioxide and more especially during recovery, the retention of potential (Levin[10]) is much altered from the normal, being increased in amplitude but probably not in duration. It lasts in altered nerves, as in the normal, for the A wave at least 200 to 300 sigma and for the C wave at least 1000 to 1200 sigma, that of the B wave approximating that of the C rather than the A.

While investigating the effects of anoxemia, etc., on the A wave it was noted (unpublished data) that the recovery curve, that is, the curve of threshold strength of stimulus against time after a first response, showed an increased height but that complete recovery was not noticeably protracted, that is, the end of the relatively refractory period remained about the same. The change in the steepness of the curve of the recovery of thresholds of the A wave above described resembles the change in steepness of the retention curve under CO_2 and anoxemia.

Definite evidence of treppe during depression as regards both the body of the action potential and its retained portion has been observed.

It is a privilege to acknowledge the helpful criticism of Doctor George H. Bishop in this work.

[8] Bishop, George H., and Heinbecker, Peter, *Am. J. Physiol.*, 1930, in press.

[9] Amberson, W. R., and Downing, A. C., *J. Physiol.*, 1929, lxviii, 19.

[10] Levin, A., *J. Physiol.*, 1927, lxiii, 113.

Pacific Coast Section.

Stanford University School of Medicine, February 12, 1930.

4822

The Effect of Pituitary Extract Upon the Absorption of Glucose and Iodide.

C. H. THIENES AND A. J. HOCKETT.

From the Department of Pharmacology, University of Oregon and the Department of Physiology and Pharmacology, University of Southern California.

In an earlier report[1] there was presented indirect evidence of a decreased rate of absorption of drugs from the alimentary tract in animals which had received subcutaneous injections of *Liquor Pituitarii.* In more recent experiments we have made use of the white rat, according to the method of Cori and Cori[2] to determine the rate of absorption of glucose and iodide. After preliminary trials, 4 series of 6 rats each were employed: (a) test animals, given 0.5 gm. glucose per 100 gm. body weight in water by stomach tube, preceded 5 minutes and 20 minutes by subcutaneous injections of 1/8 cc. *Liquor Pituitarii;* (b) controls, treated as in the preceding series, except that 0.85% NaCl, made acid with dilute HCl to the same pH as the *Liquor Pituitarii,* was used instead of the gland extract; (c) test animals given 0.5 gm. potassium iodide, per 100 gm. body weight, in water by stomach tube, preceded 5 minutes and 20 minutes by subcutaneous injections of 1/8 cc. *Liquor Pituitarii* and, (d) controls treated as in (c), but using acid-saline injections instead of pituitary extract.

The animals were killed exactly 2 hours after the administration of the glucose or iodide, the stomach and intestine removed, opened in distilled water, rinsed several times, and the washings analyzed for glucose or iodide to determine the amount unabsorbed.

Tables I and II give the results of the experiments.

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[1] Thienes, C. H., and Hockett, A. J., *J. Pharm. Exp. Ther.*, 1928, 33.

[2] Cori, C. F., and Cori, G., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 495.

TABLE I.
Mg. Glucose Absorbed Hourly per 100 gm. Body Weight.

	Controls	Test Rats Injected with Pituitary
	190	140
	222	169
	200	94
	262	143
	192	135
	195	91
	184	96
Average	206	125

TABLE II—IODIDE ABSORPTION.
Mg. Potassium Iodide Absorbed Hourly per 100 gm. Body Weight.

	Controls	Test Rats Injected with Pituitary
	86	60
	102	30
	110	43
	127	37
	98	80
	96	76
Average	103	54

Conclusions: Subcutaneous injections of *Liquor Pituitarii* in the white rat decreased the rate of absorption of glucose and of iodide from the gastrointestinal tract.

4823

The Effects of Low Glycogen Content Upon the Contraction Process in Isolated Skeletal Muscle.

PAUL W. SMITH AND MAURICE B. VISSCHER.

From the Department of Physiology, University of Tennessee, and the Department of Physiology and Pharmacology, University of Southern California.

A large series of observations has been made to check the findings of Olmsted and Coulthard,[1] who report that gastrocnemius muscles in which little or no glycogen can be detected, taken from frogs rendered glycogen poor by means of insulin convulsions, contract upon electrical stimulation, and produce fatigue curves closely resembling the normal. They also report the increase in lactic acid content in such muscles upon fatiguing to be greater than can be accounted for by the disappearance of carbohydrate.

[1] Olmsted, J. M. D., and Coulthard, H. S., *Am. J. Phys.*, 1928, lxxxiv, 610.

Analyses of the muscles of 40 normal and insulinized frogs have been made (both bull frogs and grass frogs were used). The corresponding muscles of opposite limbs were used in the determination of resting and fatigue levels. The muscles were frozen in liquid air previous to preparation for analysis. The Pflüger method, modi-

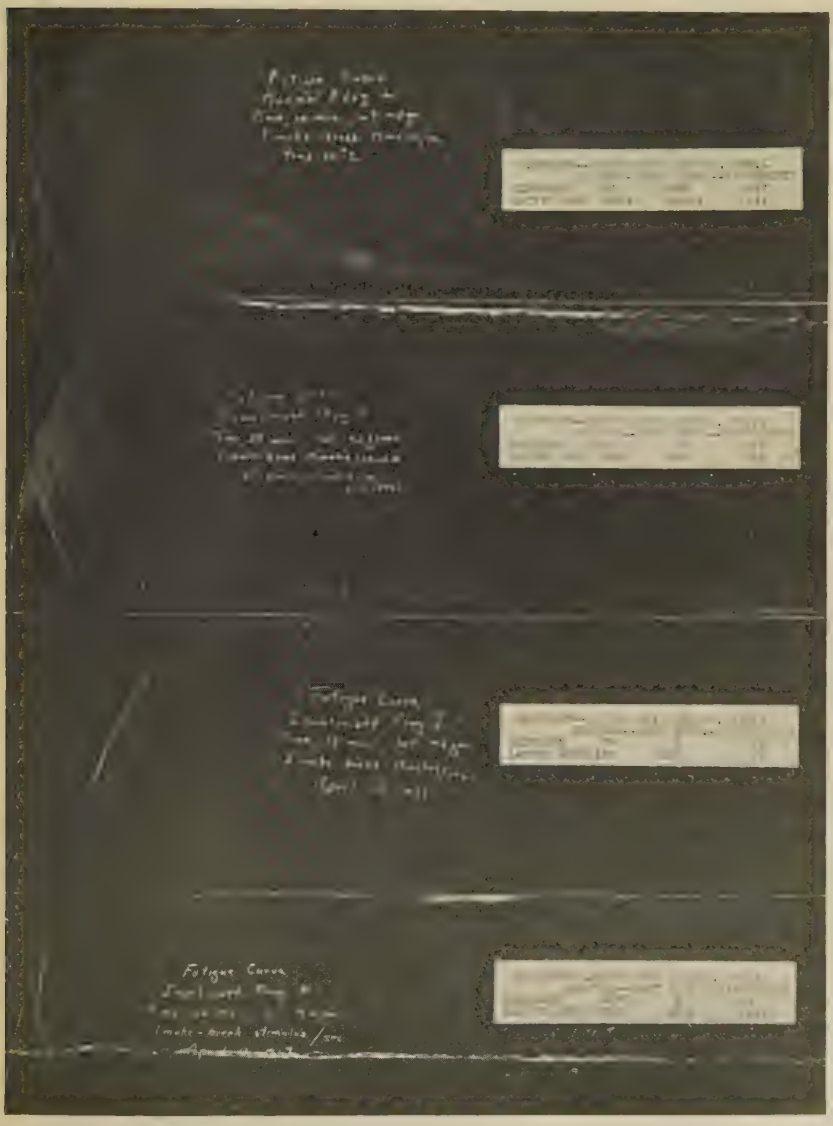


FIG. 1.
Fatigue curves, normal and insulinized frogs.

fied as suggested by Evans[2] was used in the determination of glycogen. The Friedeman, Cotonio, and Shaffer[3] method was used in the determination of lactic acid.

We have not been able to duplicate the findings of Olmsted and Coulthard. The area of the fatigue curve, at constant kymograph speed, seems to bear a direct relationship to the lactic acid produced regardless of the influence of insulin (see Fig. 1). Also, the disappearance of glycogen is closely balanced by the increase in lactic acid. In a number of cases no glycogen could be detected in the resting muscle.

In each of these cases the increase in lactic acid was sufficiently slight to have come from a resting level of glycogen below the threshold of the method used in its determination.

4824

Source of the Pigmentary Hormone of Amphibian Hypophysis.

BENNET M. ALLEN.

From the University of California at Los Angeles.

There has been some confusion as to the rôle of the different lobes of the hypophysis in the secretion of the hormone that causes the well known pigmentary effects in tadpoles consisting of a darkening in the presence of an excess of the hormone and the assumption of a very pale color in its absence. The various methods of experimentation have been largely responsible for this diversity of view. P. E. and I. B. Smith[1] caused these pigment changes by making repeated intraperitoneal injections of saline extracts of crushed beef glands, securing positive results with *pars anterior, pars nervosa* and *pars intermedia,* the last named being the most pronounced. This method would not preclude the possibility of the fainter color changes caused by *pars anterior* and *pars nervosa* material being due to the presence of secretion diffused from the *pars intermedia.*

In the work of the writer[2] for several years the method of transplantation has been used. Care is taken to use only unmixed material

[2] Evans, J. L., *Physiol. Rev.,* 1926, vi, 367.

[3] Friedeman, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.,* 1927, lxxiii, 335.

[1] Smith, P. E. and I. B., *Endocrinol.,* 1923, vii, 579.

[2] Allen, B. M., *Science,* N. S., 1920, lii, 274-276.

of the *pars intermedia* sliced off at some distance from its surface of intimate union with the *pars nervosa.* Transplants of the latter never cause pigmentary effects when taken with similar precautions against contamination with *pars intermedia* substance. In all cases the region of junction between these two portions is discarded. While there is a slight transitory pigmentary effect produced by *pars anterior* or *pars nervosa* transplantation, they do not persist, while on the other hand *pars intermedia* of an adult frog transplanted into normal or hypophysectomized tadpoles becomes functional and causes most intense expansion of the superficial melanophores with deposition of pigment granules in the epidermal cells. This forms a dense mass closely applied to the side of the nucleus directed toward the surface of the body. These changes were followed in a series of photographs of a selected group of cells in the tail of a living tadpole into which a transplant had been made. These cells followed through the course of 10 days showed a very great increase in the number and degree of expansion of the superficial melanophores while the deeper xantho-leucophores had become contracted to points and had not increased in number.

The conclusion from work of this kind repeated through several years is that the pigmentary hormone is produced only by the *pars intermedia.*

4825

Molecular Structure of Valonia Cellulose Membrane.

O. L. SPONSLER.

From the University of California at Los Angeles.

The arrangement of β d-glucose anhydrous residues in the cellulose framework of the cell-wall membrane of *Valonia* was determined by x-ray crystal structure methods. The chain molecule with the residues as units of structure is the same as that in plant fibers.[1] In the latter, the arrangement of molecules laterally with reference to the surface of the fiber is not experimentally demonstrable. In *Valonia,* however, it was readily demonstrated that the 6.10 A. u. planes of the lattice[2] are parallel to the surface of the spherical wall

[1] Sponsler and Dore, *Colloid Symposium Monograph IV,* 1926, 174-202; Mark and Meyer, *Ber. d. d. Chem. Gesells,* 1928, lxi, 593, and *Z. f. physikalische Chem.,* 1929, ii, 115.

[2] Sponsler, O. L., *J. Gen. Physiol.,* 1925, ix, 221, and 1926, 677-695.

covering of the plant; and that the 5.33 A. u. planes are radial to the sphere, that is, at right angles to the 6.10 planes. It was also readily demonstrated that the 3.93 A. u. planes are diagonals to the two just mentioned. The cellulose framework of the membrane, then, when viewed along a normal to the surface would appear as a lattice, the surface layer of which has its glucose units spaced regularly 5.33 x 5.15 A. u. and the layers parallel to the surface layer spaced 6.10 A. u.; the included angles are within 2° or 3° of right angles.

4826

Inhibition of Water Diuresis by Amytal.

ERIC OGDEN. (Introduced by J. M. D. Olmsted.)

From the Division of Physiology, University of California School of Medicine.

Recently Fee[1] has shown that the water diuresis, established in decerebrate dogs by administration of water through the stomach tube, is checked by the administration of chloroform, ether, chloralose, or morphine in the doses commonly employed to produce anesthesia or analgesia. The inhibition lasts, roughly speaking, for the same length of time as the narcotic effect.

The technique given in the paper referred to, has been followed exactly in the 6 experiments reported here with the exception that intraperitoneal amytal was used instead of the drugs previously employed.

In all cases the full dose (0.05 gm. per kilo) produced an immediate and lasting inhibition, complete as regards the excess water elimination. In the only two cases where measurements were made at minute intervals, the diminution in urine flow began during the second and third minutes respectively, following the injection of amytal. In one experiment the excretion was followed for over 10 hours and no recovery was observed.

The following are the records of 2 typical experiments, the measurements being expressed graphically:

I. Dog, male, 7.0 kilos. Anesthetized with chloroform and ether 50/50.
9:40 a. m. Decerebration complete.
10:15 " Cannulation complete.

[1] Fee, *J. Physiol.*, 1929, lxviii, 39.

11:00 " Urine flow well established. 0.5 cc. per 10 minutes.
12:10 p. m. 500 cc. water by stomach tube A.
2:45 " 4.0 cc. N/2 NaOH intraperitoneally B.
3:15 " 0.4 gm. amytal in 4.0 cc. NaOH intraperitoneally C.
2:00 a. m. (approx.) Animal died.
Urine flow: Correlate with reduced size of curve X.

II. Dog, male, 7.5 kilos. Anesthetized as above.
4:45 p. m. Anesthetized.
5:25 " Decerebration complete.
6:10 " Cannulation complete.
8:50 " Urine flow established. 0.8 cc. per minute.
9:30 " 250 cc. water by stomach tube a.
10:07 " 250 cc. water by stomach tube b.
11:23 " 0.2 gm. amytal in 2 cc. N/2 NaOH, intraperitoneally c.
12:15 a. m. 0.2 gm. amytal in 2 cc. N/2 NaOH, intraperitoneally d.
1:00 " Observation abandoned, death probably 4-5 hours later.
Urine flow: Correlate with reduced size of curve O.

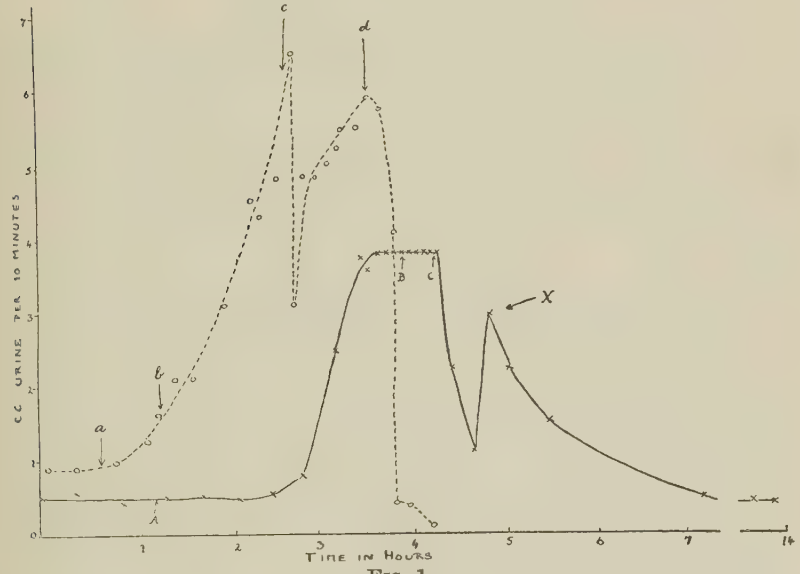


FIG. 1.

No explanation is offered for the rise in curve I (marked ×) but a similar rise was also noted in another experiment.

The partial inhibition observed after the first injection in curve II has been observed with a dose as little as 1/10 gm. in a 6 kilo dog.

4827

Studies on Permeability of Living Cells. XII. Further Studies on Penetration of Oxidation-Reduction Indicators.

MATILDA MOLDENHAUER BROOKS.

From the Department of Zoology, University of California.

In a previous paper[1] it was shown that there was a definite relation between the penetration of oxidation-reduction indicators into cells of *Valonia macrophysa* and their position in the oxidation-reduction scale. Those dyes which were at the electro-positive end of the scale penetrated very readily, while those which were farther down towards the electro-negative end did not penetrate. The former were represented by the indophenols, which were reduced in the sap; and the thiazines, which were not reduced. The latter included the indigo-sulphonates, which did not penetrate.

The question was then raised whether the position of the dye on the oxidation-reduction scale was the determining factor in its penetration. Fortunately Rapkine, Struyk and Wurmser[3] have determined the potentials of some vital dyes on the E_h scale, and found that Janus green and neutral red are more electro-negative than any of Clark's dyes; in fact the neutral red system lies very near to the H electrode. These 2 dyes were, therefore, used in further penetration studies.

About one dozen small plants of *Valonia ventricosa* which had been kept in the laboratory for some months and seemed to all outward appearances to be normal living cells, were placed in solutions of sea water containing 0.001% Janus green and buffered with Clark's buffers at various pH values from 5.8 to 9.0. The total M concentration of buffer was 0.009. The temperature was 25° C. The sap was examined at various intervals up to 12 hours and in no case was the dye found in the sap in detectable amounts either in the oxidized or the reduced state.

Other plants were placed for one hour in a solution of 0.001% Grübler's neutral red dissolved in sea water containing buffers as previously described at pH 5.8. A spectrophotometric analysis of the neutral red dissolved in sea water gave an absorption maximum at wave length of 523 mμ, which corresponds to that found by For-

[1] Brooks, M. M., *Am. J. Physiol.*, 1926, lxxvi, 360.

[2] Clark, W. M., Cohen, Barnett, and Gibbs, H. D., *Pub. Health Rpts.*, 1925, xl, 1131.

[3] Rapkine, L., Struyk, A. P., and Wurmser, R., *Compt. Rend. Soc. Biol.*, 1929, c, 1020.

manek and Grandmougin.[5] There was also a secondary maximum at about 465 mμ. Spectrophotometric analysis of the pink sap which was extracted from 5 cells kept under these conditions for one hour, gave the same absorption maxima as the dye itself. The method of extraction is described in another paper.[1] That the cells were not irreversibly injured is shown by the fact that they lived for months afterwards in the laboratory and appeared normal.

These results confirm unpublished data on the penetration of these dyes into sap of freshly collected plants of *Valonia macrophysa* which the writer obtained in 1925 while in residence at Bermuda.

These experiments show, therefore, that the position of the dye system on the oxidation-reduction indicator scale as described by Clark, is the factor determining whether or not a dye is reduced by the cell, but that it is not the exclusive factor which determines the penetration of these dyes into living cells.

4828

Actions of Sodium Bismuthate.*

CHAS. GURCHOT, JEAN SPAULDING, H. G. MEHRTENS AND P. J. HANZLIK.

From the Neuropsychiatric Division of the Department of Medicine, and the Department of Pharmacology, Stanford University School of Medicine, San Francisco, California.

The bismuth compounds in current use contain the bismuth as cation, or in basic form, and do not dependably penetrate the brain and appear in the cerebrospinal fluid. Compounds containing the bismuth as anion, or in acid form, have not been tried previously, but, on theoretical grounds, might be expected to penetrate the central nervous system more readily than those containing bismuth as cation, and thus be more valuable in neurosyphilis. Accordingly, we have explored the possibilities of sodium bismuthate and obtained results worthy of record at this time in order to indicate the desirability of further study of anionic bismuth.

[4] Clark, W. M., "The determination of H ions." Williams and Wilkens, Baltimore, 1928.

[5] Formanek, J., and Grandmougin, E., *Untersuchungen und Nachweis organischen Farbstoffe auf Spektroskopischen Wege.* (Berlin), Julius Springer, 1908.

* This research was supported in part by a grant from the Committee on Research in Syphilis.

Sodium bismuthate ($NaBiO_3$) is the sodium salt of bismuthic acid with a bismuth content of 74.5%. It is obtainable in the market as a yellowish brown powder and possesses very low solubility in all ordinary and special solvents. The solubility in water and 0.9% sodium chloride solution is about 1:10,000, and in whole serum, 1:5,000, and sufficient liberation of bismuth occurs to give a darkening with sulphide. The reaction of the watery suspension (marketed product) is strongly alkaline. Strong mineral acids and 85% lactic acid dissolve bismuthate readily, but weak organic acids (citric and acetic) only slightly.

The toxicity of bismuthate was found to be very low. Rabbits (5) tolerated doses of from 0.6 to 1 gm. per kilo intramuscularly, and mice and rats (9), from 42 to 90 mgm. per kilo hypodermically. A fatal dose has not been found. The compound has been injected as a suspension in water or in acacia solution, without evidence of irritation. Three rabbits receiving 0.018 gm. per kilo intravenously and 0.22 and 0.6 gm. per kilo intramuscularly, excreted variable though considerable daily quantities of bismuth (about 1.4 mgm.) in urine over long periods (up to 4 months), the absorption being slow and continuous. Of 8 guinea pigs, receiving 0.1 gm. per kilo intramuscularly, 4 showed the presence of unabsorbed bismuthate in the muscles till the end of 14 days, and the remaining guinea pigs showed no depots 19, 21 and 33 days after administration. All of these guinea pigs showed presence of bismuth in the brain, amounting to a median of 0.09 mgm. Bi per 100 gm. of brain after a sojourn of the bismuthate during from 5 to 33 days in the body. Five rabbits receiving from 18.5 to 47.6 mgm. bismuthate (13.8 to 35.5 mgm. Bi) per kilo intravenously showed the presence of bismuth in the brain in from 3 to 24 days after injection. A definite antisyphilitic action of the bismuthate was demonstrated in the experimental syphilis of rabbits as follows: Prevention of chancres occurred in a pair of rabbits receiving 0.032 and 1 gm. per kilo during 3 and 6 months, respectively, while the untreated, control pair showed fully developed lesions with spirochetes at the end of 16 to 30 days. In curative experiments on developed chancres, in pairs of rabbits, 0.4 gm. per kilo of bismuthate caused clearing of lesions at the end of 72 hours and healing at the end of 21 days, whereas the untreated controls healed at the end of 41 days. As further controls, treatments with bismuth sodium tartrate and neoarsphenamine, in both prophylactic and curative experiments, gave the usual beneficial results.

Thus, anionic bismuth possesses a destructive action on the syphi-

litic virus, just as does the cationic form, with the advantages of cerebral penetration and comparatively low toxicity, but a more soluble compound than the bismuthate would be desirable.

4829

A Demonstration of the Curability of Malignancy in Rats by a Low Pressure Environment.

E. S. SUNDSTROEM AND G. GIRAGOSSINTZ.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Previous workers[1] who have carried out experiments on the effect of lowered oxygen tension on malignant neoplasms have reported a retarded growth and an extensive necrosis but no positive cures. They evidently missed the time when the factors which ultimately may annihilate the cancer reach their highest potency. Our success is attributable to (1) an adequate period of exposure, (2) a gradual adaptation to successive steps of decreasing oxygen tension, and (3) an uninterrupted maintenance of the final pressure throughout the experiment. We are not prepared to emphasize the superiority of our method, the reduction of the total pressure, as compared with the adjustment of the oxygen tension at normal atmospheric pressure. We believe, however, that it is technically more convenient and that it affords a greater margin of safety.

Our low pressure chambers are improved models of the apparatus described previously by one of us.[2] The pressure is controlled automatically and is disturbed neither by any manipulation in the care of the animals nor by the removal and the replacement of individual rats. A series may therefore be continued for almost an unlimited time, although the maximal exposure time in our cancer work has been 6 weeks exclusive of the one week adaptation period. Our results for the first 2 weeks have closely corresponded to those of other investigators and we shall, therefore, in this report consider mainly those series which have lasted longer.

We have employed 2 pressure levels: 300 and 360 mm., corresponding in altitude to 25,000 and 20,000 feet respectively. With

[1] Warburg, O., *et al.*, *Klin. Wochenschr.*, 1926, v, 829. Campbell, J. A., and Cramer, W., *Lancet*, 1928, ccxiv, 828.

[2] Sundstroem, E. S., and Bloor, W. R., *J. Biol. Chem.*, 1920, xlv, 155.

a few exceptions, adapted rats continue to do quite well in both of these environments and we believe that even the lower pressure level would also be endurable to patients. When the gradual adaptation time is omitted, on the other hand, one half of the rats succumb at the 300 mm. level. It appears from our statistics for adapted cancer rats that the mortality has been relatively high but these figures include a number of preventable deaths. The majority of the deaths have been due to accidents, for instance, irregularity in the ventilation, or to unrecognized ailments of the lungs, or to the choice of unsuitably large tumors for the treatment. Such tumors disintegrate very rapidly, rupture and become infected. The deaths of these rats have probably been due to the absorption of noxious material. Very few of such cases have been completely cured.

The experiments have been carried out on the Flexner-Jobling carcinoma and the Jensen sarcoma. We have observed a few spontaneous retrogressions occurring in very small tumors of the former type but never when they had reached the size we employed in our earlier low pressure work. On the other hand, we have noted 2 retrogressions in our sarcoma controls with respect to tumors which were equally large (15-30 mm. in diameter) as those we have treated in our later work. We were informed by Dr. C. F. Cori of the Cancer Institute in Buffalo, who was kind enough to supply us with breeders (and also with the sarcoma strain*) that retrogressions are very infrequent in this strain of rat.

We have inoculated successfully nearly 300 rats of the aforementioned strains of neoplasms. After deducting the numbers of such cancerous rats which we have used as controls, and of those which were treated for shorter periods or used for special experiments, we have tabulated the results for the remaining 133 rats which were given a treatment of from 3 to 6 weeks. The data are summarized in Table I. The term "definitely cured" is used for cases in which the animals were observed for periods of 2 to 6 months after their removal from the tanks. The tumors were considered to have been "apparently cured" in cases in which the crucial test of extended observation was not or could not be applied, for instance, with respect to dead rats, but in which the histological examination indicated a complete necrosis of the neoplasm.

In addition to the experiments involving low pressure at ordinary room temperature we have also considered the possibility that a raised temperature (usually 35° C.) could act as an adjuvant to the

* We are indebted to Dr. F. C. Wood at the Cancer Institute of Columbia University for the carcinoma strain.

low pressure in annihilating malignancy. It appears that the histological picture of tumors in this latter series is altered in such a way so as to suggest that the combined environmental method of cancer treatment may prove advantageous. The "tropical mountain climate" appears to lessen the mortality of the rats. Since it is yet doubtful whether the combined treatment affects the percentage of cures we have not entered any subdivisions based on temperature in this report.

TABLE I.

Type of Tumor	Pressure	Number of Rats	Mortality	Definite Cures	Apparent Additional Cures
	mm.		%	%	%
Carcinoma	300	32	28	34	22
	360	33	21	24	12
Sarcoma	300	29	14	83	14
	360	39	38	38	13

These data indicate a correlation between the destructibility of the malignant growth and the pressure level used and, on the other hand, a greater vulnerability of the sarcoma strain. We believe, however, that the seemingly speedier response of the latter strain as compared with the carcinoma may be partially due to the inequality in the size of the tumors which we used for the 2 series. The results of the "sarcoma, 300 mm." series are, furthermore, almost too good to be reproduceable. It is probable that in this series some unknown factor, for instance of seasonal origin, may have assisted the low pressure in annihilating the cancer.

Ten rats were inoculated with sarcoma a few minutes before they were placed in the tank. Contrary to our expectation that the tumor fragments would fail to grow we obtained 100% takes. The tumors underwent the same phases of growth and subsequent necrosis as tumors which were already large at the start. Such results are difficult to interpret under the assumption that the reduced oxygen tension is only directly responsible for the destruction of cancer in low pressure environments. One is tempted to surmise that the reduction of the oxygen tension may also act in an indirect way, for instance, by effecting a diminution of some substance which is essential for the growth and maintenance of the cancer or an accumulation of some substance, known or unknown, which is destructive to the tumor. In our studies of the time relations in the levels of certain blood constituents, for instance with respect to the sugar and non-protein nitrogen, we have obtained results which suggest a parallelism with the time relations in the effectivity of the low pressure treatment on cancer.

One would expect no lasting effect of the oxygen deficiency if its action were only direct. On the other hand, if it produces some biochemical modifications which are injurious to malignant growths, these modifications might affect also a cancer which is inoculated subsequent to the cessation of the low pressure exposure. One of our more recent experiments seems to prove that malignancy acquired by pretreated rats may run a different course than in untreated animals.

Eleven normal rats were exposed to a 300 mm. pressure and inoculated with sarcoma on the first or second day after their removal from the tank. The control series comprised 9 rats which were inoculated at the same time with fragments from the same sarcoma. The takes were in both series 100%. The tumors grew at first at an equally rapid rate. Later 3 of the pretreated neoplasms retrogressed completely, as compared with one retrogression among the controls. The other control tumors grew to a huge size and presented an almost solid mass of normal sarcoma tissue. On the other hand, the tumors of the pretreated animals became very soft and the section of them showed that they had liquified, leaving only a thin rim of cancer tissue of doubtful normality.

4830

A Comparison of Ultrafilterable Serum Calcium and Cerebrospinal Fluid Calcium in Humans.*

DAVID M. GREENBERG. With the Assistance of H. E. Ballard and J. B. Dalton.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Cameron and Moorhouse[1] have proposed using the cerebrospinal fluid calcium as a measure of the diffusible calcium of the blood plasma, arguing that the choroid plexus as a living, colloid impermeable membrane gives us a more perfect distribution of the diffusible constituents of the blood than can be obtained in any *in vitro* manner. This implies both that the cerebrospinal fluid is formed by a process of diffusion rather than of active secretion and also that the fluid is continuously in diffusion equilibrium with the constit-

* Presented at the Ann Arbor meeting of the Society of Biological Chemists, April, 1928.

[1] Cameron, A. T., and Moorhouse, V. H. K., *J. Biol. Chem.*, 1925, lxiii, 687.

uents of the plasma. Whether the cerebrospinal fluid is formed by an active process of secretion or is simply a dialysate of the blood plasma is still a mooted question.

Histological data and evidence from the mechanics of circulation, favoring the dialysis theory have recently been reviewed by Fremont-Smith.[2] It has been pointed out by Updegraff, Greenberg and Clark[3] and by Fremont-Smith,[2] that the published data of various authors for the amounts of uric acid, urea, glucose, magnesium and inorganic phosphate present in plasma and spinal fluid respectively are difficult to reconcile with the diffusion theory.

Even if it is granted that the spinal fluid is a dialysate of the blood plasma, there still remains the important point of whether the spinal fluid is continuously in equilibrium with and gives a true picture of the changing states of the constituents of the blood plasma, or if it is only rarely in actual diffusion equilibrium with the blood plasma. If there is diffusion equilibrium, the distribution of the ionic constituents between plasma and spinal fluid is expected to conform to the relations governing a Donnan membrane equilibrium. Hamilton[4] arrived at the conclusion: " . . . . an equilibrium of the Donnan type may, at least partly, govern the distribution of electrolytes between serum and spinal fluid, but it seems probable that the equilibrium is modified by unknown factors." Van Slyke[5] takes the viewpoint that the deviations in the electrolyte distribution from the Donnan theory are probably due to the spinal fluid not being usually in diffusion equilibrium with the blood plasma. Further, anatomical considerations hardly seem to favor the view that the bulk of the spinal fluid is continuously in diffusion equilibrium with the blood coursing through the capillaries of the choroid plexus. In view of the conflicting findings and opinions it seems that a great many more experimental data will be required before the questions of the manner of origin and the relationship between spinal fluid and blood can be definitely settled.

Experimental: To test the hypothesis that the spinal fluid calcium is a measure of the diffusible calcium, we have carried out parallel determinations of the diffusible calcium and the spinal fluid calcium on blood and spinal fluid obtained simultaneously from human subjects. The diffusible calcium was determined by the analysis of

[2] Fremont-Smith, F., *Arch. Neurol. and Psych.*, 1927, xvii, 317.

[3] Updegraff, H., Greenberg, D. M., and Clark, G. W., *J. Biol. Chem.*, 1926, lxxi, 87.

[4] Hamilton, B., *J. Biol. Chem.*, 1925, lxv, 101.

[5] Van Slyke, D. D., "Factors affecting the distribution of electrolytes, water and gases in the animal body." Harvey Lectures, 1926.

serum ultrafiltrates, the ultrafiltration being carried out according to the procedure described by Greenberg and Gunther.[6] The values obtained for total calcium, diffusible calcium and spinal fluid calcium on the 9 subjects available are given in Table I. It is unfortunate that more subjects were not available to obtain a more extensive series of results and thus a more rigorous test of the relation between the diffusible and spinal fluid calcium. However, since work on the problem has been discontinued for the present, it seems desirable to publish the data obtained rather than put off publication in the hope of obtaining a larger series of results at some future time.

TABLE I.

A Comparison of Diffusible Calcium and Cerebrospinal Fluid Calcium.*

Name	Diagnosis	Total Ca mg. per 100 cc. Serum	Diffusible Ca mg. per 100 cc. Ultrafiltrate	Spinal Fluid Ca mg. per 100 cc.	R= Ca S.P. / Ca d
Cro.	Post influenza encephalitis	10.5	6.5	5.1	0.78
Cal.	Diagnosis not established. Spinal fluid globulin 3+	8.1	3.7	5.2	1.40
Pit.	Mitral stenosis	10.3	4.9	5.1	1.04
Geo.	Urethral calculus	8.3	4.3	5.0	1.16
Jor.	Acute myocitis	10.6	5.7	5.1	0.89
Sne.	Syphilis	9.6	4.2	5.0	1.19
Ahe.	Syphilis. Spinal fluid globulin 3+		4.8	4.9	1.02
Dav.†	Infant		4.3	4.5	1.04
Eis.	Syphilis	9.6	4.4	4.3	0.98

* The blood and spinal fluid samples were obtained through the courtesy of Drs. Lewis Gunther and Wm. J. Kerr of the Department of Medicine, University of California Medical School.

† Blood obtained by heel puncture.

It is to be noted that all the subjects presented various pathological conditions although none were definitely known to be suffering from a disease in which calcium metabolism is known to be disturbed. The use of pathological cases is an advantage for the experimental test to be made in the present instance. For under such circumstances greater fluctuations of the blood constituents and thus a better chance to detect lags in the maintenance of equilibrium with the spinal fluid are to be expected. The diagnoses of the cases studied are given in the table.

If there is an equilibrium between blood and spinal fluid, differences between the obtained values for diffusible calcium and spinal

[6] Greenberg, D. M., and Gunther, L., *J. Biol. Chem.*, 1930, lxxxv, 491.

fluid calcium are to be expected because of the use of serum rather than plasma and because of the Donnan distribution. However, a rather constant ratio between the 2 calciums should be obtained if this idea is correct. The results obtained show that in 4 of the cases, the difference between diffusible and spinal fluid calcium was not marked, being little more than the probable error of the analytical method. The differences in the other 5 cases are considerable. The variations are indicated by the ratios of spinal fluid calcium to diffusible calcium given in the last column of Table I. The values for spinal fluid calcium obtained are in good agreement with the figures published by Hamilton[4] and others. Of the values for diffusible calcium, the figure for subject "Cal," 3.7 mg. per 100 cc. of ultrafiltrate is markedly lower than the normal level and 6.5 mg. per 100 cc. for "Cro" is high. For subject "Cal" the total calcium is also concomitantly low. The other values are within the normal limits for diffusible calcium.

The results of Table I point to the conclusion that when the blood calcium is at a normal stable level there is an approximate approach to an equilibrium between blood and spinal fluid and the spinal fluid calcium is then a close measure of the diffusible calcium. But on the other hand, when the blood constituents are undergoing marked fluctuations the spinal fluid changes do not keep pace. This hypothesis offers an explanation of the anamolous findings of Cameron and Moorhouse[1] who on parathyroidectomized dogs obtained but little lowering of the spinal fluid calcium although the total calcium dropped greatly. The hypothesis of a near approach to equilibrium in cases of a normal stable level of the blood constituents and the failure to keep pace with marked fluctuations also harmonizes with the approximate agreement to a Donnan membrane distribution found in some instances and the wide departure in others noted by Hamilton.[4]

4831

Effects of Typical Broncho-Dilating Drugs on Intrapleural Pressure.*

SELLING BRILL AND CHAUNCEY D. LEAKE.

From the Surgical Thoracic Clinic and the Pharmacological Laboratory of the University of California Medical School, San Francisco.

By means of suitable trochars and rubber tubing, one may observe intrapleural pressures from both sides of the chest of normal unanesthetized dogs. The tubing may be branched so that both manometric and kymographic records may be obtained for intrapleural pressure changes from each side of the chest. With such an arrangement we have noted the effects on intrapleural pressure of the administration of (1) epinephrin, a typical stimulant of the thoraco-lumbar autonomics and (2) atropine, a drug acting by peripheral paralysis of the cranio-sacral autonomics. Both drugs cause bronchodilatation.

Examples of approximate intrapleural pressure changes in dogs following the administration of these drugs are offered in the tables. The mean pressure, which is given in a separate column, is the average of the intrapleural pressure at inspiration and expiration. It is difficult to obtain satisfactory manometric readings with a rap-

TABLE I.

Dog—Wt. 6 kilos. No anesthesia. Intrapleural pressure readings after injection of 2 cc. epinephrin hydrochloride 1/1000 subcutaneously.

	Right Chest		Left Chest	
	IPP in cms. of H_2O	Mean IPP in cms. of H_2O	IPP in cms. of H_2O	Mean IPP in cms. of H_2O
Normal average	+0.6 to —8.4	—3.9	+2.0 to —8.6	—3.3
5 min. after epinephrin	+2.6 to —8.4	—2.9	+3.4 to —8.6	—2.6
8 min. after epinephrin	+2.6 to —8.8	—3.1	+3.4 to —8.2	—2.4
9 min. after epinephrin	+3.6 to —8.4	—2.4	+3.2 to —8.2	—2.5
15 min. after epinephrin	+3.6 to —9.0	—2.7	+4.2 to —8.2	—2.0
17 min. after epinephrin	+3.6 to —8.4	—2.4	+3.8 to —8.2	—2.2
24 min. after epinephrin	+4.4 to —8.8	—2.2	+5.8	
29 min. after epinephrin	+4.0 to —8.8	—2.4		
1 hr. after epinephrin	+5.6 to —7.8	—1.1		

* Supported in part by grants from the J. J. and Nettie Mack and the Purington Research Funds.

TABLE II.

Dog—Wt. 9 kilos. Anesthesia sodium neonal, 450 mgm. intraperitoneally. Intrapleural pressure readings after intravenous injection of 0.4 cc. epinephrin hydrochloride 1/1000.

	IPP Right Chest in cms. H_2O	Mean IPP in cms. H_2O
Normal	—3.6 to —7.0	—5.3
3 min. after epinephrin	—3.6 to —7.2	—5.4
6 min. after epinephrin	—3.4 to —7.2	—5.3
8 min. after epinephrin	—2.6 to —6.6	—4.6
10 min. after epinephrin	—1.6 to —5.6	—3.6
11 min. after epinephrin	+0.2 to —5.6	—2.7
13 min. after epinephrin	+3.0 to —5.6	—1.3
15 min. after epinephrin	+5.4 to —5.6	—0.1

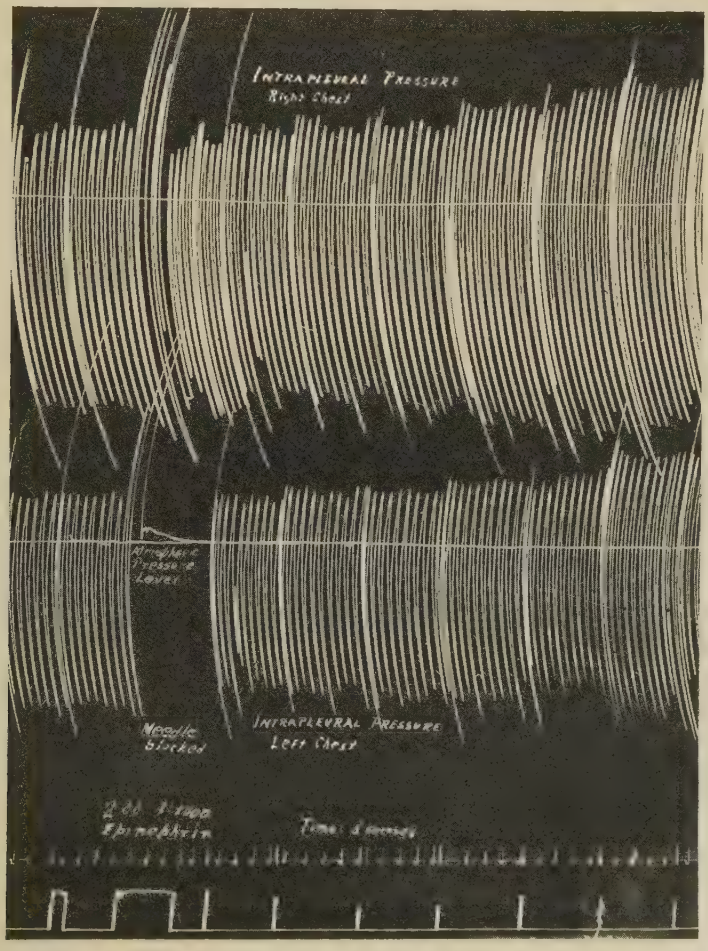


FIG. 1.

Kymographic record showing increase in intrapleural pressure following the subcutaneous injection of 2 cc. 1:1000 solution of epinephrin hydrochloride.

TABLE III.

Dog—Wt. 18 kilos. No anesthesia. Intrapleural pressure readings after injection of atropine sulphate subcutaneously.

Jan. 9. Dose 6 mg.			Jan. 31. Dose 5 mg.		
	IPP Left Chest in cms. H_2O	Mean IPP in cms. H_2O		IPP Left Chest in cms. H_2O	Mean IPP in cms. H_2O
Normal	—0.8 to —5.8	—3.3	Normal	—2.0 to —6.0	—4.0
2 min. after atropine	—0.8 to —5.2	—3.0	3 min after atropine	0.0 to —6.0	—3.0
5 min. after atropine	—0.8 to —5.2	—3.0	6 min. after atropine	+1.0 to —7.0	—3.0
8 min. after atropine	+0.6 to —4.8	—2.1	10 min. after atropine	—1.0 to —6.6	—3.8
11 min. after atropine	+2.8 to —4.8	—1.0	14 min. after atropine	—1.4 to —6.0	—3.7
13 min. after atropine	+5.2 to —2.8	+1.2	17 min. after atropine	—0.6 to —6.6	—3.6
15 min. after atropine	+5.2 to —2.8	+1.2	20 min. after atropine	+1.4 to —6.0	—2.3
			23 min. after atropine	+0.6 to —5.0	—2.2

idly moving column of water and, therefore, the kymograph tracings probably present a better picture of the changing intrapleural pressure.

Examination of the kymographic tracings and of the data presented in the tables shows that the administration of epinephrin or

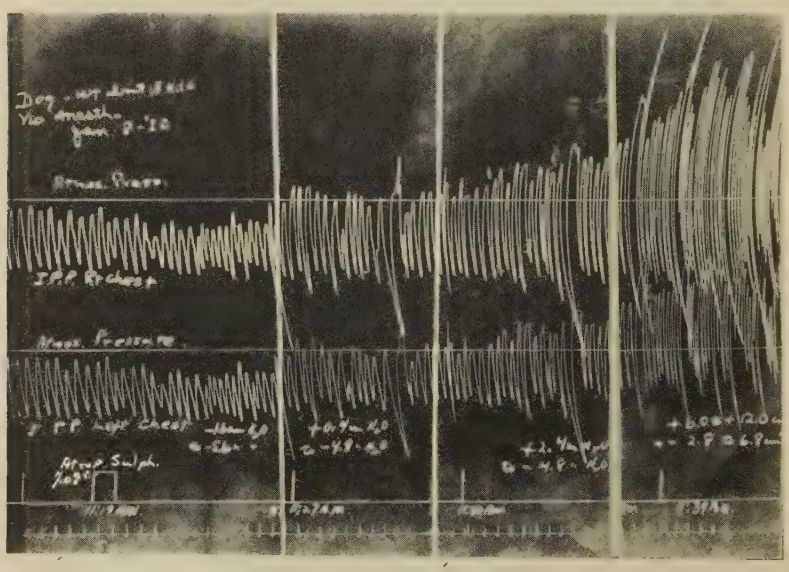


FIG. 2.

Kymographic record showing increase in intrapleural pressure following the subcutaneous injection of 6 mgm. atropine sulphate, in an unanesthetized dog.

atropine is followed by a definite increase in intrapleural pressure. The effect seems quite prolonged, and in the case of epinephrin, does not become apparent until the action of the drug on blood pressure has practically disappeared. With broncho-dilatation, less resistance is offered to the movement of air in and out of the lungs and this seems to be accompanied by less suction on expansion of the chest.

4832

Effect of Emetine Hydrochloride by Subcutaneous Injection on Oxygen Consumption in Human Subjects.

MEI-YU CHEN AND H. H. ANDERSON.* (Introduced by C. D. Leake.)

From the Pharmacological Laboratory of the University of California Medical School, San Francisco, and the Pacific Institute of Tropical Medicine.

Pellini and Wallace[1] in their review of the pharmacology of emetine mention the cardiac depressant action of the drug with the resultant fall in blood pressure. Respiratory changes may also occur but were thought to be dependent upon circulatory failure. These workers noted in fasting dogs an increase in total nitrogen, urea nitrogen, and ammonia nitrogen following injections of emetine. These results were believed to be due to an interference with intracellular metabolism with an accompanying acidosis. No work appears in the literature on the effect of emetine on oxygen consumption.

We have attempted to determine the effect of emetine hydrochloride† in therapeutic doses on pulse rate and pressure, respiratory rate, and oxygen consumption in normal humans and in patients under treatment for amebiasis. Five medical students were selected as "normal" subjects and 3 clinic patients were used for study, 2 harboring *Entamoeba histolytica* and one with gall-bladder disease. The oxygen consumption tests were made with the Sanborn "graphic" apparatus by the closed method.

After a 15-hour fast period and after lying quietly for 30 minutes the patient's pulse rate and pressure, and respiratory rate were taken and oxygen consumption tests were made. Following this the drug

* Lilly Research Fellow.

1 Pellini, E. J., and Wallace, G. B., *Am. J. Med. Sci.*, 1916, clii, 325.

† Ampoules of emetine hydrochloride containing 0.065 gm. (Eli Lilly and Co.).

was administered in total doses of 33 to 65 mgm. by subcutaneous injection. Within the hour following administration of the drug, other determinations were made on pulse, respiration, and oxygen consumption.

TABLE I.
Maximum Functional Changes During One Hour's Observation Following Subcutaneous Injection of Emetine HCl in Humans.

Subject	Sex	Age yrs.	Ht. cm.	Wt. kg.	Total Dose in mgm.	Resp. per min.	Pulse per min.	Pulse Pressure mm.Hg.	Oxygen Consumed in cc. per min.
H. S.	M	23	172.5	66.0	33	+2	—4	+10	+23
J. J.	M	21	160.0	56.0	33	0	—6	0	—10
H. A.	M	27	172.5	68.0	33	+4	0	+16	0
T. M.[1]	M	46	165.0	61.5	33	0	+3	0	—21
E. J.	M	22	175.0	66.0	65	0	+5	0	— 5
C. W.	M	26	165.0	61.0	65	0	0	— 6	—37
O. R.[2]	F	28	151.0	54.4	65	0	+9	—13	—20
T. M.[1]	M	46	165.0	61.0	65	0	0	0	—10
J. A.[1]	M	24	160.0	52.3	65	+2	0	—10	—19
J. A.[1]	M	24	160.0	52.3	65	0	0	0	— 5
J. A.[1]	M	24	160.0	52.3	65	+8	0	0	— 4
J. A.[1]	M	24	160.0	52.3	65	+2	0	0	—35
J. A.[1]	M	24	160.0	52.3	65	+5	0	0	+ 7
J. A.[1]	M	24	160.0	52.3	65	0	0	—12	— 6
J. A.[1]	M	24	160.0	52.3	65	+2	—4	0	+10
J. A.[1]	M	24	160.0	52.3	65	0	0	0	—15

[1] Infested with amoeba. [2] Gall-bladder disease.

Table I shows the results of this study. Sixteen experiments were made on 8 subjects. There was a significant fall in blood pressure in one subject, T.M., on continuous daily administration. Before treatment was begun his blood pressure was 134/90 and at the end of 10 days after having had 455 mgm. of emetine hydrochloride his blood pressure was 114/76. J.A. also exhibited a fall in blood pressure from 106/76 to 86/66 in 10 days after a total of 520 mgm. of the drug. His "normal" basal metabolic rate dropped from zero to —12 during this time.

Where a change occurred in respiratory rate it was increased. Changes in pulse rate were slight and inconstant. Pulse pressure dropped in 4 cases and was elevated in two. Oxygen consumption was generally lowered from 4 to 37 cc. per minute. In 3 cases increases of 7, 10, and 23 cc. per minute were noted. None of the subjects exhibited symptoms of nausea or vomiting following injections of the drug.

Summary: In 8 subjects on which 16 experiments were carried out a slight depression of oxygen consumption was noted following subcutaneous injections of therapeutic doses of emetine hydrochlo-

ride. Respiration was increased when affected. Changes in pulse rate were inconstant. A definite fall in blood pressure was noted in 2 instances when 455 to 520 mgm. were given over a 10-day period.

4833

The Formation of Lactic Acid Following the Administration of Glucose and Fructose.

M. I. ROSE, G. GIRAGOSSINTZ AND E. L. KIRSTEIN.
(Introduced by J. M. D. Olmsted.)

From the Division of Physiology, University of California Medical School, Berkeley, California.

Campbell and Soskin[1] and Campbell and Maltby[2] have shown that the high R. Q.'s following the administration of dihydroxyacetone as reported by Himwich, Rose and Malev[3] could in great part be ascribed to "extra" CO_2 released from bicarbonate by blood lactic acid (blood drawn from arm vein) which parallels the rise in quotient. Campbell and his coworkers found that the increase in lactic acid follows fructose as well as dihydroxyacetone. On the other hand, aldose sugars such as glucose, galactose, etc., produce no such lactic acid changes. Inasmuch as the source of lactic acid arising from ketose substances has not been accounted for, we have undertaken a research in that direction. Simultaneous samples of blood from the portal vein, the hepatic vein, femoral artery, and femoral vein were drawn and analyzed for lactic acid and reducing sugar immediately before and at half hour intervals following the injection into the small intestine of 25 gm. of fructose in 125 cc. water, and in other experiments after 25 gm. of glucose. Amytal anesthesia was used.

At this time we wish to call attention to a rise of between 50% and 100% of lactic acid in the portal blood over and above resting values in all fructose experiments. Glucose brings about little, if any, change in lactic acid in the portal blood. In other words, lactic acid seems to be produced in the region of the abdominal viscera when fructose is given by way of the intestinal tract. The lactic

[1] Campbell, W. R., and Soskin, S., *J. Clin. Invest.*, 1928, vi, 291.

[2] Campbell, W. R., and Maltby, E. J., *J. Clin. Invest.*, 1928, vi, 303.

[3] Himwich, H. E., Rose, M. I., and Malev, M. R., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 238.

acid values of blood drawn from the hepatic vein are considerably lower than the high portal values, indicating removal of the lactate by the liver.

Further work is now in progress to determine whether the lactic acid actually arises in the gut as a breakdown product of fructose.

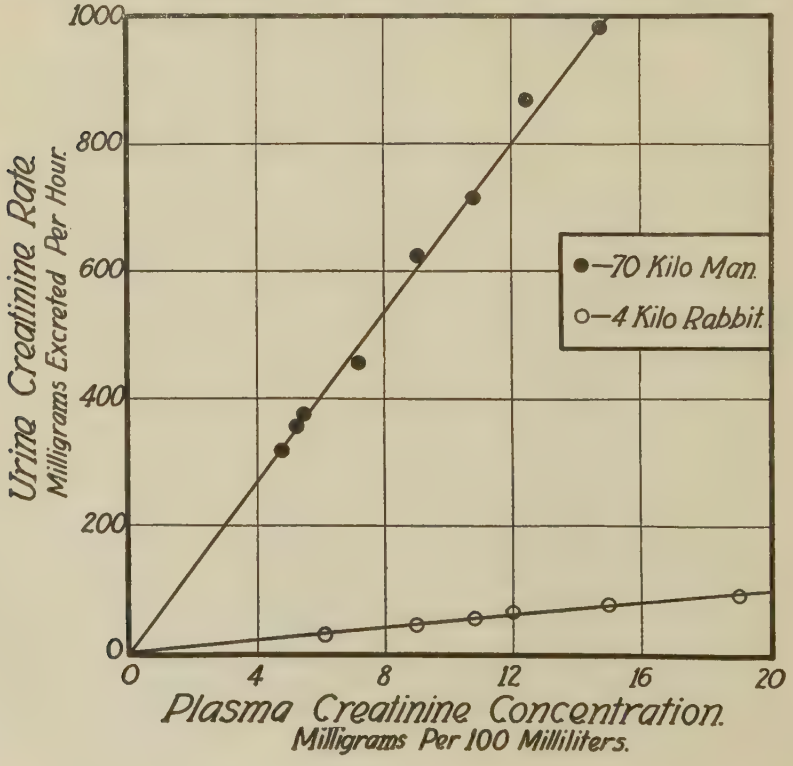
4834

Relation of Creatinine Excretion in Urine to Plasma Creatinine Concentration.

EATON M. MACKAY.

From the Scripps Metabolic Clinic, La Jolla, San Diego, California.

Following the administration of creatinine to normal rabbits during a copious diuresis, under conditions most apt to lead to full renal activity, the rate of creatinine excretion in the urine has been found to be directly proportional to the plasma creatinine concentra-



tion. A similar relationship has been found in man. Typical examples are presented in Fig. 1. The large difference in the values of the ratios: $\frac{\text{Urine rate}}{\text{Plasma concentration}}$ is undoubtedly due, as in the case of urea,[1] to the different amounts of renal tissue possessed by man and the rabbit. Under the same conditions urea behaves in a like manner[2] although the urine rate for a given plasma concentration is always greater for creatinine. That is, the excretory ratio $\frac{\text{Urine rate}}{\text{Plasma concentration}}$ is less for urea. The significance of this will be discussed elsewhere. Since the difference between the ratios for these 2 substances appears to bear a constant relation to the lower it is obvious that creatinine may be substituted for urea in measuring renal function by Addis' method.[3] Rehberg[4] has used creatinine in what is essentially this method but failed to observe the standard or other constant conditions and obtained inconstant results. He found[5] only a general tendency for the urine rate to increase in proportion to the plasma concentration. An examination of the method using creatinine under more rigid conditions is now in progress.

4835

Bacteriostatic Action of Dyes on the Organisms of Undulant Fever.

M. S. MARSHALL AND DOROTHY JARED.

From the Department of Bacteriology, University of California Medical School, San Francisco.

As one of the various means purported to differentiate the undulant fever bacteria of caprine, bovine, and porcine types, the inhibitory effect of certain dyes, particularly gentian violet and thionin, has been suggested. As with other means of differentiation, there is no absolute scale for comparison, inasmuch as not all strains fall consistently into any one group by all differentiating tests.[1] The present study attempts to throw some light on the discrepancies in so far as the bacteriostatic effect of dyes is concerned.

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[1] Taylor, F. B., Drury, D. R., and Addis, T., *Am. J. Physiol.*, 1923, lxv, 55.

[2] Addis, T., and Drury, D. R., *J. Biol. Chem.*, 1923, lv, 105.

[3] Addis, T., *Arch. Int. Med.*, 1922, xxx, 378.

[4] Rehberg, P. B., *Zentralbl. f. inn. Med.*, 1929, l, 367.

[5] Rehberg, P. B., *Biochem. J.*, 1926, xx, 447.

[1] Meyer, K. F., and Eddie, B., in press.

The technic, briefly described, consisted of making appropriate dye concentrations in melted liver hormone agar, from which plates were poured, and cultures streaked on a sector from a broth suspension of an agar slant culture, to insure a degree of uniformity in inoculum. The results were most clearly observed after 72 to 96 hours of incubation at 37° C. The cultures used were obtained from Tunis, Austria, Italy, Denmark, Germany, and from the western part of the United States, and represented a variety of animal and human sources.

The correlation between the original sources of cultures and the bacteriostatic effect of gentian violet, basic fuchsin, brilliant green, thionin, and methylene blue, considered individually or collectively, was found to have a number of discrepancies. Gentian violet in dilutions of 1:50,000 allowed growth with some strains, and inhibited completely in a 1:250,000 dilution with others. Basic fuchsin, chemically related to gentian violet, in most instances allowed virtually normal growth in a 1:50,000 dilution, or even lower, but in some instances inhibited completely in a dilution of 1:100,000. Brilliant green possessed a marked inhibitory quality, permitting on occasion a moderate growth in a 1:1,250,000 dilution; other cultures were almost entirely inhibited in a dilution twice as great. Methylene blue and thionin, although chemically related, vary greatly in bacteriostatic action. Methylene blue with most strains completely inhibited in a dilution of 1:1,000,000, although some growth occurred with several strains. Thionin, on the other hand, permitted vigorous growth in a dilution of 1:25,000 in some instances, although a 10-fold dilution inhibited other strains.

Four samples of thionin, from 3 manufacturers, showed a marked divergence in bacteriostatic action. For example, 40% of the strains tested were completely inhibited in a 1:250,000 dilution of one dye sample, whereas another sample showed no inhibition in any instance in this dilution. The latter required a concentration approximately 5 times as great in order to inhibit in the same percentage of cultures tested as the former sample, and the inhibited strains were not the same.

The quantity of inoculum influences the degree of inhibition by dyes. It was found possible to obtain growth in dye concentrations 5 to 10 times as great as those ordinarily inhibiting by the use of a reasonably heavy inoculum from a slant culture.

The specific stability of bacteriostatic action shows little, if any, variation, as tested with the same cultures over a period of one year.

Among other apparently demonstrable vagaries in dye inhibition of this group of organisms, perhaps one of the most important is the stage of development of the particular strain. The rough, or R, type of colonial growth appears in most instances tested to be definitely more resistant to bacteriostatic action than does the smooth, or S, type. Comparative growth of S and R growth on the same dye plates frequently reveals striking differences. Inasmuch as the S and R forms, although relatively stable, are at no time perfectly stable, this seems an additional source of difficulty or of error in the utilization of bacteriostatic action as a means of type differentiation.

Illinois Section.

Northwestern University, February 25, 1930.

4836

The Etiological Rôle of Bacteria in Bile Peritonitis. An Experimental Study in Dogs.*

ALLAN G. REWBRIDGE AND L. S. HRDINA. (Introduced by Edmund Andrews.)

From the Department of Surgery of the University of Chicago.

According to the prevalent view "bile peritonitis" is due to the toxicity of bile. Horrall[1] observed that when bile was allowed to drain continuously into the peritoneal cavity the dogs died within 24 hours. He attributed the cause of death to the toxicity of the bile salts. In order to gather additional data on the mechanism of "bile peritonitis" the following experiments were performed.

In a series of 20 dogs, peritonitis was produced by allowing bile to drain into the peritoneal cavity. Determinations of bilirubin by the Van den Bergh method and bile salts by the quantitative Pettenkofer reaction developed by Aldrich[2] were made on blood drawn from the femoral veins of these dogs, 4 and 18 hours after their operations. No increase of bilirubin or bile salts could be detected by these methods even though the animals were dying as the result of their peritonitis. At necropsy the peritoneum was acutely inflamed, the surfaces covered with a thin layer of fibrin and a few small areas of fat necrosis were observed around the pancreas. The peritoneal cavity contained a serosanguinous exudate in which were observed polymorphonuclear leukocytes and Gram positive bacilli. This organism was cultured from the peritoneal exudates of all of the 20 dogs studied. The source of this bacillus was the next problem to be solved. Was it entering the peritoneal cavity with the

* This work has been done under a grant from the Douglas Smith Foundation of Medical Research of the University of Chicago.

1 Horrall, O. H., *Arch. Int. Med.*, 1929, xliii, 114.

2 Aldrich, M., and Bledsoe, S. M., *J. Biol. Chem.*, 1928, lxxvii, 519.

bile or was bile altering the permeability of the viscera containing bacteria to permit them to invade the peritoneal cavity?

Cultures of bile removed from the gall bladder at the time of the operation were all sterile except one in which grew a short Gram negative bacillus. A 10% solution of bile salts, filtered through a Berkefeld filter, and shown to be sterile when introduced into the peritoneal cavity produced a peritonitis identical with "bile peritonitis" except that fat necrosis was more extensive. Of the 20 dogs in this experiment smears and cultures of the peritoneal exudate showed the same Gram positive bacillus in 19. In one no growth occurred.

Twenty cubic centimeters of an 18 hour broth culture of this bacillus when introduced into the peritoneal cavity produced a peritonitis identical with "bile peritonitis" except that areas of fat necrosis were absent. From the peritoneal exudate the same gram positive bacillus was cultured.

The bacillus, a strictly anaerobic organism, grows readily in broth and produces stormy fermentation within 18 hours in milk. The colony is large on an anaerobic blood agar plate, varies its color from yellow to brown and is surrounded by a wide zone of beta hemolysis. It stains well with methylene blue and positively by Gram's method. It varies considerably in length, is broad, square ended and has an occasional subterminal spore. In smears it appears singly, in pairs or in short chains. This organism is either *B. welchii* or some other bacillus closely related to it.

These observations show that bile peritonitis is an infection which is produced by *B. welchii* or some other anaerobic bacillus closely related to it.

4837

The Variation of Anesthetic Efficiency of Procaine Hydrochloride and Procaine Borate With pH.

LEONARD S. FOSDICK, HAROLD L. HANSEN AND CARL A. DRAGSTEDT.

From the Department of Chemistry of the Dental School and the Department of Physiology and Pharmacology of the Medical School, Northwestern University, Chicago, Illinois.

Experimental evidence obtained both in the laboratory[1] and in the clinic[2] shows that the borate of diethylamino ethyl p-amino-benzoate,

[1] Benedict, Dailey and Arnim, *Dental Cosmos*, 1929, lxxi, 866.

[2] Freeman, *Dental Cosmos*, 1929, lxxi, 949.

procaine borate, has a higher anesthetic efficiency than the hydrochloride of the same base. The fact that the solutions of procaine borate used had a pH of about 8.4 while the procaine hydrochloride solutions had a pH of approximately 5.6 suggested that it might be worth while to determine the effect of variation of pH on the anesthetic efficiency of the two drugs.

O. Gros[3] found that addition of excess sodium bicarbonate or sec-

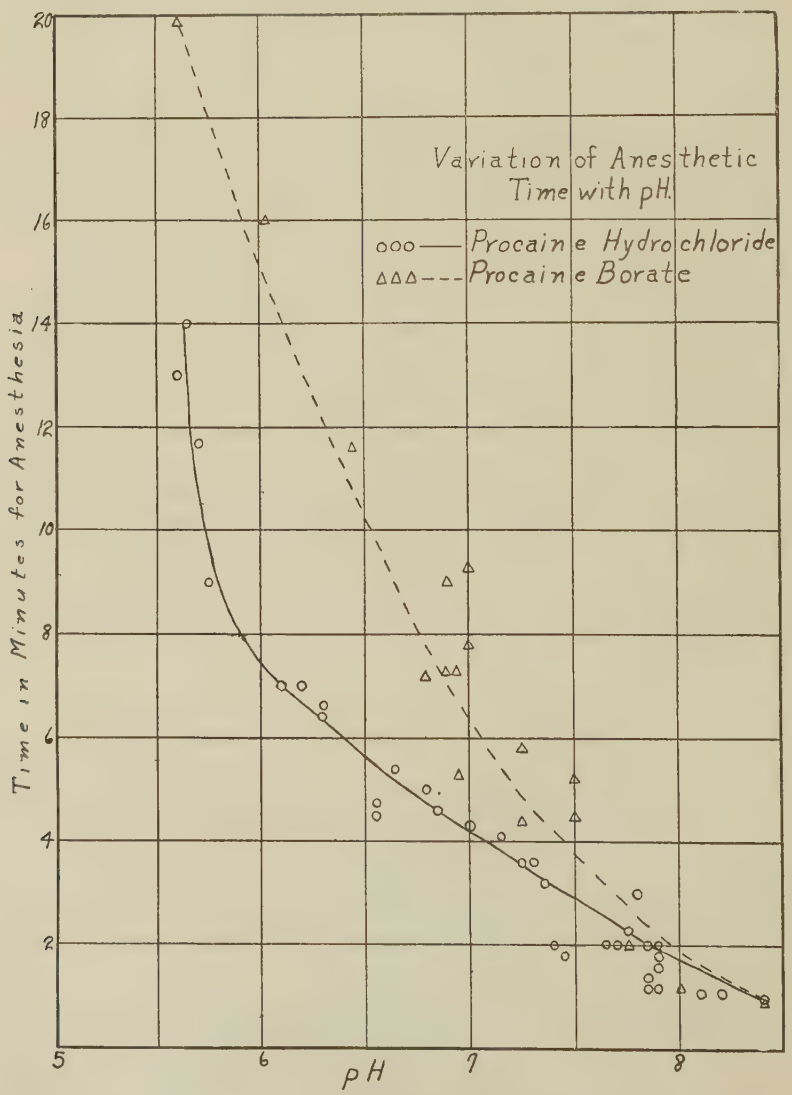

FIG. 1.

[3] Gros, *Arch. Exp. Path. Pharm.*, 1912, lxvii, 132.

ondary sodium phosphate markedly increases the anesthetic efficiency of procaine hydrochloride. Sollmann[4] corroborated and extended these findings. Regnier,[5] from his results on alkalinization of cocaine hydrochloride solutions, also concluded that increase in alkalinity augments anesthetic properties.

The present work consisted in a comparison of the anesthetic efficiency of procaine borate and procaine hydrochloride solution at varying hydrogen ion concentrations by the method of Adams[6] on goldfish with modifications suggested by Dailey[7] and Benedict. The solutions used were brought to the desired pH with N/10 sodium hydroxide and N/10 hydrochloric acid. The pH determinations were made with a Leeds-Northrup Quinhydrone potentiometer. All solutions used were of the same concentration with respect to the anesthetic base, the procaine hydrochloride solutions containing 1 gm. of the hydrochloride in 500 cc. of solution and the procaine borate solutions containing 1.638 gm. of the borate in 500 cc. of solution.

The results obtained are given in the graph. Each point represents the average time required to anesthetize 3 or more fish, anesthesia being determined by lack of response to stimulation of fins or tail. As can be seen by the curves the efficiency as determined by the time for anesthesia varies for both solutions and at a given pH is approximately the same for each drug. Apparently the increased efficiency of procaine borate is due to its alkalinity. Observations on the anesthetic effect of the 2 solutions on the rabbit's cornea confirm these findings on the goldfish.

4838

Effect of Diet on the Healing of Experimental Gastric Ulcer.

G. B. FAULEY AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Ferguson[1] was able to produce uniformly in rabbits gastric ulcers which persisted from 2 to 8 months or longer. He incised the an-

[4] Sollmann, *J. Pharm. Exp. Therap.*, 1917, x, 379; *J. Am. Med. Assn.*, 1928, lxx, 216.

[5] Regnier, *Compt. rend. de biol.*, 1925, xcii, 605.

[6] Adams, Rideal, Burnett, Jenkins, Dreger, *J. Am. Chem. Soc.*, 1926, xlviii, 1758.

[7] Dailey and Benedict, *Dental Cosmos*, 1929, lxxi, 704.

[1] Ferguson, *Am. J. Anat.*, 1928, xlii, 403.

terior wall of the stomach and at the point of incision removed a piece of mucosa and then closed the stomach by a silk suture, the rabbits being kept on a diet of hay, oats and carrots. It occurred to us that this observation provided a method of studying the effect of diet on the healing of this experimental gastric ulcer.

In our first series of rabbits we found that if a lesion was made in the posterior wall of the stomach in which no silk suture was present and one in the anterior wall in which a silk suture was present and the rabbits placed on the stock diet of hay, oats and carrots, that the posterior lesion healed in 30 days, but the anterior lesion did not. This showed that the silk suture was a factor in delaying healing and that in the absence of the silk, diet played no rôle in delaying healing.

Anterior lesions of the Ferguson type were made in 29 rabbits. Twelve were placed on the stock diet and 17 on a diet of milk, bread and mashed boiled carrots. The rabbits were sacrificed on the 30th day. All of the 12 rabbits on the "rough diet" had ulcers at the 30th day. Only 3 of the 17 on the "soft diet" had ulcers. The results show that the silk suture *per se* is not sufficient to prevent the ulcer from healing and that a "rough diet" plus the silk suture factor are sufficient to produce a chronic gastric ulcer, grossly and histologically, and that a "soft diet" favors the healing of gastric lesions.

The same results were obtained in a series of 4 rabbits on a "rough diet" in which a gut suture was used instead of silk.

In another series of 4 rabbits which were kept on a diet of dry "quick rolled oats" with a fiber content of only 1.4%, the ulcers failed to heal. On opening the stomachs of the animals, the contents were found to be as pasty and dry as that found on the "rough diet." This indicates that the fluidity of the gastric contents is also a factor determining the healing of gastric lesions.

Experiments are under way to test further the fluidity factor as well as fat and food-buffer factors.

4839

Effect of Insulin on Alimentary Lipemia in Normal Dogs.

H. R. RONY AND T. T. CHING. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

The blood fat curve following ingestion of a test meal of fat was studied by us on adult dogs. The fat meal consisted of 2 cc. olive oil per pound body weight by stomach tube. Venous blood was removed before and at certain intervals after the meal, and the plasma analyzed for total fatty acids and cholesterol by Bloor's new method. The test meals were given after 7 to 14 days' fasting. Such dogs invariably responded with a marked increase in blood fat beginning about 2 hours after the test meal, reaching a peak in about 5 hours and returning to the previous level in 10 hours.

In a series of 8 dogs, 20 to 40 units insulin were given hypodermically at the same time the fat meal was given. The result was that the amount of the total fatty acids remained practically unchanged. This shows that the insulin prevented alimentary lipemia from occurring.

We then determined the effect of glucose administration on the blood-fat curve in connection with a fat meal. In 5 experiments, 1 gm. of glucose per pound body weight in 20% solutions was given by stomach tube along with the olive oil to dogs starved from 7 to 14 days. Again the blood fat remained practically unchanged in all cases, demonstrating that oral administration of glucose prevents the alimentary lipemia.

TABLE I.

Dog No.	Fasting Days	Amt. of Insulin Units	Total Fatty Acids Mgm.		
			Before	2 hrs.	5 hrs.
102	8	20	305	314	310
103	10	30	289	268	304
105	7	25	410	425	432
106	14	25	324	316	318
107	10	30	194	176	205
108	12	40	343	340	338
109	8	30	259		270
110	11	25	428	415	402

This suggests that the effect of insulin on alimentary lipemia is in some way connected with the effect of insulin on the carbohydrate metabolism. Whether both insulin and glucose prevent ali-

mentary lipemia by increasing the glycogen content of the liver is yet to be proven.

4840

The Support Reaction in Spinal Animals.

S. W. RANSON AND J. C. HINSEY.

From the Institute of Neurology, Northwestern University Medical School, Chicago.

Rademaker observed what he called the "Stütz" reaction in decerebellate dogs. Pressure against the pads of the toes, simulating that exerted by the floor against the foot, causes contraction of the muscles of the entire leg in such a way as to convert it into a prop or Stütz. This reflex is a very important factor in reflex standing. Since its discovery by Rademaker,[1] it has been described in detail by Schoen[2] and Pritchard.[3]

Pressure against the pads of the foot stimulates the nerve endings in the skin (exteroceptive) and by stretching the muscles which flex the toes and extend the ankle it also stimulates the nerve endings in these muscles (proprioceptive). Both of these types of stimuli take part in producing the reflex. According to Schoen it occurs after section of all of the nerves to the skin of the foot and must then be purely proprioceptive. In decerebellate animals it can be evoked by touching the pads of the toes, which shows that it can be elicited by tactile stimuli acting alone.

It is most easily studied in animals in which all of the brain in front of the thalamus has been removed. Schoen was unable to demonstrate it in spinal animals except that there was some indication of its presence in decapitate animals when the neck reflex was exerting an influence favorable to extensor tonus.

We have observed the support reaction in decapitate dogs, decapitate cats, and in both acute and chronic low spinal cats. In acute experiments with decapitate and low spinal preparations the reflex can best be demonstrated when reinforced by extraneous stimuli. Pinching the tip of the tail will often furnish the required reinforcement. We have studied the animals when supported in a hammock with the legs hanging pendant through 4 openings in the support.

[1] Rademaker, G. G. J., *Dtsch. Z. f. Nervenheilk.*, 1926, xciv, 144.

[2] Schoen, R., *Pflüger's Arch. f. d. ges. Physiol.*, 1926, ccxiv, 21 and 48.

[3] Pritchard, E. A., Blake, *Pflüger's Arch. f. d. ges. Physiol.*, 1926, ccxiv, 148.

When supported in this way, a decapitate dog resembles a decerebrate dog. The Stütz-tonus is so strong that the hind quarters of the animal can be lifted off the hammock by pressure against the pads of the hind feet, and the legs will bear the body weight for a minute or more. We believe that the pressure of the hammock upon the skin of the abdomen and groin reinforces the support reaction in the hind legs. If the animal is placed upon its back the rigidity disappears. This reinforcement seems to be sufficient in the dog. In the decapitate cat, additional reinforcement from pinching the tail is required; but when reinforced in this way, the support reaction becomes strong and steady and enables the hind legs to carry the body weight for minutes at a time.

In chronic spinal cats with transections at various levels from the third lumbar to the fourth thoracic segments the support reaction becomes within a few days after the operation sufficiently strong to enable the animal to stand. The hind legs bear the weight of the posterior half of the body when the animal is out of the hammock and standing on a table. In our experiments, the length of time which the hind legs of chronic spinal cats have been able to bear the body weight in the standing posture has varied from 30 seconds to 3 minutes and 20 seconds. Standing of a similar sort was seen by Sherrington[4] in chronic spinal dogs.

4841

Production of Lenticular Opacities by Ultraviolet Radiation in the Presence of Certain Salts.

MARIE A. HINRICHS.

From the Department of Physiology, University of Chicago, Chicago, Ill.

The results of Hess[1] were confirmed by the production of a surface destruction of the lens epithelium following exposure of the eyes of frogs to ultraviolet radiation. Burge[2] showed that lens material in a test tube was more readily coagulated if salts or sugar were present, because the lens protein was so modified that radiations of short wave-length could precipitate it. He was also able to produce cataracts in the eyes of fish which had been kept in salt

[4] Sherrington, C. S., *J. Physiol.*, 1910, xl, 28.

[1] Hess, C., *Arch. f. Augenheilk*, 1907, lvii, 185.

[2] Burge, W. E., *Am. J. Physiol.*, 1914, xxxvi, 21.

solutions for varying periods of time before they were exposed to ultraviolet radiation. (Burge.[3])

In our experiments, 3 types of procedure were followed: exposure of excised lenses in salt solutions, exposure of the eyes of intact living frogs to direct radiation, in some cases, following the injection of salt solutions into the dorsal lymph sac, and exposure of the lenses of developing chick embryos. In each case, the unscreened radiation of a Cooper-Hewitt quartz mercury-vapor arc was used.

In the first series, the excised lenses of dogs, chickens, and a frog, were exposed directly to the radiation in 0.1% and 1.0% solutions of NaCl and $CaCl_2$. Controls of lenses exposed in Ringer's solution and of unexposed lenses in salt solution were used. The results of these experiments may be summed up as follows: the lenses of dogs exposed for 22-50 minutes at a distance of 25 cm. from the center of the arc, in 1.0% NaCl showed no change in opacity, but slight surface degeneration appeared. (These dogs had been anesthetized for use in other experiments.) The chick material consisted of lenses of young chicks and adult hens, and was used immediately after removal from the animals, except in 2 cases where the material was kept on ice for a few hours before exposures could be made. These lenses were not as reactive as fresh material. Exposures were made in solutions of 0.1% and 1.0% of NaCl and $CaCl_2$ for periods of 5-23 minutes, at a distance of 20 cm. It was found that Ca was more effective in producing opacity than Na. Frog lenses similarly exposed showed opacity and surface destruction of epithelium.

In the second series of experiments, the left eyes of frogs were exposed for 3-5 minutes at 20 cm. distance from the arc. Frogs were killed and lenses examined at varying intervals after exposure. Examinations were made in tap water, in Ringer, and in salt solutions. In tap water, no opacity appeared at first, although prolonged immersion in tap water, (30-45 min.) often showed a difference in opacity between exposed and unexposed lenses. Such a difference appears in lenses shown in Fig. 1.

If solutions of salts in concentrations of 0.1%-1.0% are added, opacity appears more rapidly in the radiated lenses. It was also found that the effects of radiation did not appear during the first day, and that they became progressively more marked as time after radiation increased, up to within 10 days after exposure.

A modification of the above method involved the injection of 2 cc. of the same concentrations of salt solutions into the dorsal lymph sac of the frog about 30 minutes before exposure of the left

[3] Burge, W. E., *Abstract Bull., Nela Res. Lab.*, 1917, i, 290.

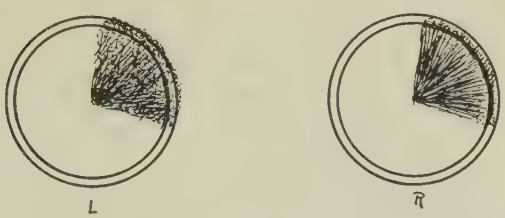

FIG. 1.
Left (L) and Right (R) lenses kept in tap water for 30 min. following exposure of left eye of frog to ultraviolet radiation. Note loss of striations in radiation left eye, as well as destructive action on lens epithelium.

eye to radiation. Controls, uninjected, and some injected with Ringer were used. It was found that the controls showed a surface effect of radiation on the lens epithelium, and that the difference in opacity could be shown by immersing both eyes in salt solutions. Radiated eyes showed more opaque lenses. However, the exposed lenses of frogs injected with salt solutions showed varying degrees of opacity detectable by gross and microscopic examinations and by the ability to focus a beam of light directed through them. Fig. 2 shows a less severe opacity but greater localized surface destruction.

The experiments with chick embryos involve the use of "point" radiation focussed through a quartz rod. A window is cut in the shell and shell membrane of the egg, and exposures are made by placing the tip of the rod directly over the developing lens. After the exposure has been made, the shell window is replaced, and sealed with paraffin. The egg is then placed in the incubator for further development. These experiments are not yet completed, but on the basis of results so far obtained (involving about 12 doz. eggs) it may be said that lens development may be inhibited. Operated and unoperated unexposed controls are used in these experiments.

In the first 2 series of experiments, involving the use of excised lenses, and of eyes of intact living frogs, it appears that opacity is due to the combined action of radiation and the combination of salts

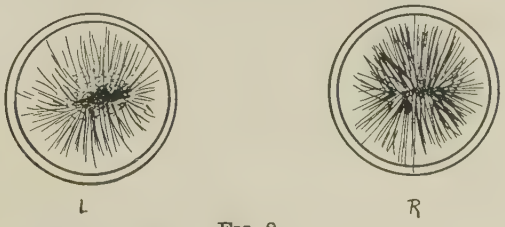

FIG. 2.
Left (L) and Right (R) lenses of frog which had been injected with 1 cc. of a 0.1% solution of CaCl. Left eye exposed 3 min. at 23 cm. Note localized area of destruction.

and lens proteins. On the basis of work done in this laboratory, and elsewhere, it seems highly probable that the primary effect of radiation is a surface phenomenon which involves the change in permeability to salts or their ions, with a subsequent precipitation of lens proteins.

I am indebted to Drs. R. S. Lillie and A. B. Luckhardt for suggestions in the study of this problem. The work was supported in part by a grant from Dr. T. J. Williams of Chicago, and in part by a grant from the radiation fund of the National Research Council.

4842

Roentgen Ray Visualization of Spleen Following Injection of Emulsions of Halogenated Oils.*

W. S. KEITH AND D. R. BRIGGS. (Introduced by P. R. Cannon.)

From the Department of Surgery of the University of Chicago.

In view of the well known phagocytic properties of the cells of the reticulo endothelial system for finely divided foreign materials injected into the blood stream an attempt was made to visualize organs containing these cells by injecting particulate matter which was relatively opaque to roentgen rays.

Using gum acacia as an emulsifying agent an emulsion of lipiodol was prepared. A few of the droplets of oil were larger than erythrocytes in the best emulsion which could be prepared by trituration in a mortar. This emulsion was diluted with 5% gum acacia solution and injected into one of the tail veins of white rats. Doses varying from ½ cc. to 4 cc. of oil per kilo body weight were given. Seven rats were so injected. Four receiving smaller amounts survived indefinitely. One died about 36 hours after injection and one died within one minute of injection. The third was killed when in a dying condition. Because of the more recent findings of Crandall and Walsh[1] we subsequently used a gum acacia-water emulsion of the bromidized esters of olive oil. This emulsion was very kindly supplied by the Abbott Laboratories.

Eleven rats weighting from 200 to 300 gm. have received intravenous doses of this material in amounts varying from ½ cc. to

* This work was done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Crandall and Walsh, *Radiol.*, 1929, xii, 499; unpublished work.

3 cc. per kilo body weight. All have survived. Six of them, 2 months later, have received a second dose. The emulsion was diluted

FIG. 1.

This rat received between 3 and 4 cc. of lipoidol per kilo body weight on 10/25/29. The oil was given as a rather imperfect emulsion, in 2 doses, each of 5 cc. of fluid and 10 minutes apart. The animal was killed, when in a dying condition, on 10/28/29. X-ray was taken 1 hour after injection.

in physiological saline and given in a volume of 5 cc. or less and in ½-1 minute. The minimum amount for visualization of the spleen in the rat would appear to be in the neighborhood of 1 cc. of oil per kilo body weight. The shadow appears to reach maximum intensity ½ hour after injection. The animals show slight respiratory disturbance and flushing of the skin of the ears for a few hours after injection. They appear normal the next day except in the cases of those receiving 2 cc. per kilo body weight or over. These animals appear normal on the second or third day. The shadow fades and disappears entirely within about one week, although at this time there appears to be still some oil left in the spleen. The liver shadow is slightly intensified following the injection.

A few similar experiments have been done on dogs. The long strap-like spleen can be visualized following the injection of about 1.5 cc. of oil per kilo body weight. By rapid injection or by using a concentrated emulsion it is easy to produce death by acute edema of the lungs. Further experiments are to be conducted using a more perfect emulsion.

We would like to call attention to the work of Radt.[2] His announcement appeared while this work was being done. He was able to visualize the spleen and liver in rabbits by injecting a coloidal solution of thorium dioxide.

The authors wish to thank Dr. P. R. Cannon for valuable advice and help, and the Abbott Laboratories for courtesy and cooperation in supplying materials.

4843

Absence of Axis Deviation of Electrocardiogram in Acute Heart Dilatation Following Experimental Embolism with Metallic Mercury.*

WILLIAM C. BUCHBINDER.

From the Cardiographic Laboratory of the Michael Reese Hospital and the Nelson Morris Institute for Medical Research, Chicago.

The injection of 5 cc. of metallic mercury into a leg vein of adult unanesthetized dogs produces a marked dilatation of the right heart chambers a very few minutes after reaching the right heart and

[2] Radt, Paul, *Klin. Woch.*, 1929, viii, 2128.

* Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

arterial side of the pulmonary circuit. There is a great disproportion between the objective symptoms and the severity of the lesions wrought in the thoracic viscera. The first symptoms that usually follow the above procedure are gastro-intestinal in nature; vomiting and defecation occur, usually within one-half hour or hour after the administration of the metal. Tachypnea or dyspnea rarely appear early, and frank respiratory symptoms are usually in abeyance for some hours. The survival period of more than 20 animals averages 35 hours.

The electrical axes computed from synchronous leads and from single axial leads that satisfy the Einthoven formula do not show significant axis deviation. There is an anomalous wide splitting of

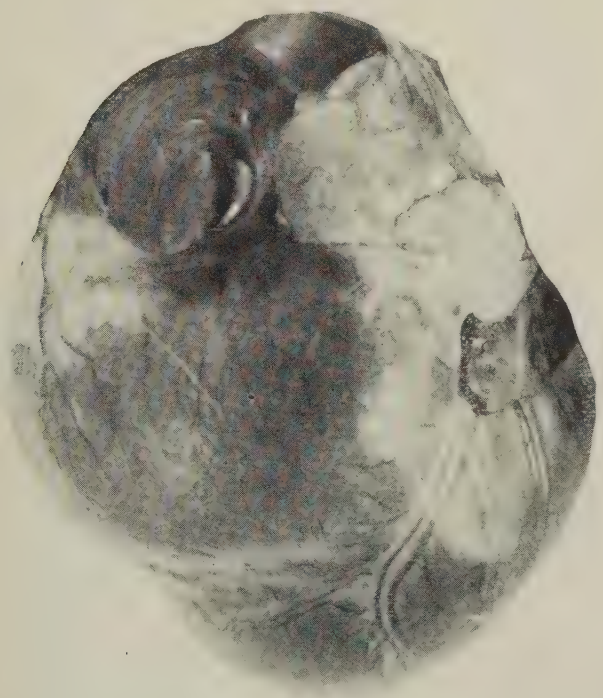

FIG. 1.

Very marked dilatations of the right heart chambers 8 min. after the intravenous administration of 5 cc. metallic mercury. Weight of dog 27.7 kilos. Heart weight 202 g. Amount of mercury recovered from right heart 0.241 g. Animal killed by intravenous ether to lessen struggle.

the T-wave in many curves. Paroxysmal auricular fibrillation was noted in the electro-cardiograms of several animals.

The relatively heavy weight of the metal in the right heart hardly enters as a factor in the interpretation of the curves as the heart empties itself usually quite completely of the metal after a few minutes. It is thought that the acute heart dilatation results from a partial obstruction in the arterial side of the pulmonary circulation.

4844

Reflexes from the Gall Bladder to the Heart.*

WILLIAM C. BUCHBINDER.

From the Cardiographic Laboratory of the Michael Reese Hospital and the Nelson Morris Institute for Medical Research, Chicago.

The sudden release of bile obtained by incising the gall bladder of a decerebrated or ether-anesthetized frog is almost invariably attended by an abrupt change in the rate and character of beating of the heart. The first event is a transient arrest of the entire heart lasting between 1 and 10 sec., almost always followed by a sinus bradycardia lasting from ½ to 10 min. Subsequently there is a return to the initial rate of beating although in a few instances progressive slowing, leading to excessive dilatation and permanent arrest, have been observed. Not infrequently the first event to be noted is a transient acceleration which precedes the slowing. The heart appears to beat much more forcibly with the inception of the slower rate. The latent interval for the reflex is a fraction of a second to a second or more.

Electrocardiograms made from base-apex leads show the cessation of activity of the sinus and ventricular portions of the heart followed by increased amplitude of R and a rather characteristic inversion of T. That the changes in the initial and final ventricular complexes are not directly associated with the reflex are to be found in the repetition of electrical effects, following an occasional sinus block which appears spontaneously after the resumption of a normal rate of beating. Such an effect is quite comparable to aberrant complexes following premature beats in mammalian electrocar-

* Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation, and in part by a grant from the Douglas Smith Foundation of the University of Chicago.

diograms. Prolongation of the PR interval does not occur and an extra-systolic arrythmia cannot definitely be determined in the electrograms.

Atropinization, decapitation or section of the vagi prevent the reflex.

In the frog there is a specific reflex from the gall bladder to the heart which appears to have a vagal origin. Katz[1] has suggested that the characteristic inversion of T with the inception of a slower rate of beating may well be a vagal effect producing asynchronous cessation of electrical effects in a ventricle in which there is decreased conduction. Irritation of the gall bladder by thermal or other instrumental means does not produce the succession of events noted when the stimulus is adequate. Acute pressure changes in the extra-hepatic ducts are thought to constitute an adequate stimulus for the production of the reflex. It has also been suggested[2] that this may be the mechanism operating for the production of arrythmias frequently seen in the human with so-called gall bladder disease, especially cholelithiasis.

4845

Fibrillation and Augmented Contractile Response of the Tongue Following Strophanthin and Digitalis.*

WILLIAM C. BUCHBINDER.

From the Cardiographic Laboratory of the Michael Reese Hospital and the Nelson Morris Institute for Medical Research, Chicago.

The response of the dog tongue to minimal break shocks applied to the intact or divided peripheral end of the hypoglossal nerve in the neck is greatly increased following the administration of digitalis-tincture or strophanthin. There is usually a fairly progressive increase in the response following the administration of the tincture preparation by way of the femoral vein. Injected into the lingual artery, fibrillation of that side of the tongue almost invariably follows and may persist for 30 minutes or an hour. Large doses applied this way may at first produce a heightened response to electrical

[1] Katz, L. N., personal communication.

[2] Buchbinder, William C., *Arch. Int. Med.*, 1928, xlii, 743.

* Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation, and in part by a grant from the Douglas Smith Foundation of the University of Chicago.

stimulation with a subsequent failure. The injection of 1 mgm. of strophanthin by way of the femoral vein produces a powerful and usually acute response of the tongue. Spontaneous fibrillation of a coarse grade lasting almost an hour has been observed after the administration of this drug without nerve stimulation.

The action of digitalis bodies upon the tongue appears to be different from that upon ordinary striped muscle. Cushny[1] has pointed out that the more recent investigators find that cardiac glucosides only weaken the muscle, reduce its excitability, quicken the onset of fatigue, and finally paralyze it completely. The similarity between the pharmacodynamic effects of digitalis bodies upon both tongue and heart is an observation that further stresses certain morphologic and physiologic comparisons drawn between the two organs in a recent communication.[2]

4846

Organ Distribution of Leucocytes.

E. F. MÜLLER, W. F. PETERSEN AND ROSE HÖLSCHER.

From the University Policlinic, Hamburg, and the Department of Pathology, University of Illinois, College of Medicine, Chicago.

Interest in the leucocytic participation between periphery and internal organs, dating from the early work of Goldscheider and Jacob[1] was revived by the publications of Widal[2] on the "hemoclastic crisis."

In our own work of this field, we were first interested in determining the participation of individual organs because of the many clinical questions so related.[3] We observed a distinct balance existing between the peripheral organs and the splanchnic group (splanchno-peripheral balance).[4] This balance is presumably one that depends on the proper functioning of the autonomic nervous system whereby peripheral vaso-constriction (leucopenia) is associated with

[1] Cushny, Arthur R., The Actions and Uses in medicine of Digitalis and its Allies. Longmans, Green and Co., London, 1925.

[2] Buchbinder, William C., *Am. Heart J.*, 1930, xci, 654.

[1] Goldscheider and Jacob, *Z. fur Klin. Med.*, 1894, xxv, 373.

[2] Widal, F., *Presse Medicale*, 1920, xxviii, 893.

[3] Müller, F. E., and Petersen, W. F., *Klin. Wschr.*, 1926, v, 53.

[4] Petersen, W. F., Müller, E. F., and Boikan, Wm., *J. Infect. Dis.*, 1927, xli, 405.

splanchnic vaso-dilatation (leucocytosis), the leucocytes accumulating chiefly in the liver. There seems very little doubt that the accumulation of leucocytes takes place in the organs of greatest metabolic activity. This having been established, a local leucocytosis justifies the assumption of increased organ activity.

In the continuation of this particular problem, we have carried out a number of operative procedures whereby individual organs were exposed under conditions that would be associated with least vascular disturbance. Using such animals, we proceeded to bring about diametrically opposite changes in the leucocyte partition, on the one hand using intravenous injections of bacteria with a resulting peripheral leucopenia,[5] and on the other hand producing peripheral leucocytosis by means of insulin shock.[6] In such animals we constantly checked the partition by means of leucocyte counts of skin and liver blood.

We have found that the leucocyte count of the vessels of the muscles, lungs, heart, brain and kidney usually corresponded to the direction taken by the skin vessels, in other words, a leucopenia was present in these vascular beds when the skin was also presenting evidence of a leucopenia. Conversely, during such conditions the liver had a relative leucocytosis and with the liver were associated the pancreas, spleen, stomach and gastro-intestinal tract.

Technically, it is of importance to note that in counting the leucocytes of the vessels of the splanchnic region, distinct differences appear in the number of leucocytes following rapidly repeated counts. If, for instance, one makes a superficial cut in the liver or spleen and then takes one leucocyte count after another the second drop usually contains fewer leucocytes than the first—one may have a count of 14,000, then 8,000, and then 6,000. It is only when we make a small fresh incision each time that we get values that correspond to the first count. This technical procedure must always be observed when experiments of this type are made; needless to say, pressure or torsion or any other unusual trauma on an organ promptly changes the leucocyte count. Presumably, these rapid changes that occur with repeated withdrawal of blood from the same region are due to a very rapid reactive local vaso-constriction of the involved region.

Under the conditions described, we have found that the leucocyte count of the abdominal organs under conditions of splanchnic stimulation (peripheral leucopenia) is highest in the liver and spleen and

[5] Müller, E. F., and Petersen, W. F., *Z. f. d. ges. Exp. Med.*, 1929, lxvi, 442.
[6] Müller, E. F., and Petersen, W. F., *Klin. Wschr.*, 1926, v, 1025.

somewhat lower in the stomach and gastro-intestinal tract. Conversely, with a relative splanchnic leucopenia, we found the fewest number of leucocytes in the liver, while those of the spleen and gastro-intestinal tract were usually equal.

In view of the fact that the vessels of the skin are accompanied by parallel changes of the musculature, brain, kidney and mediastinal organs with an opposite orientation in the splanchnic region, we believe that additional support is given the assumption that the Widal hemoclastic crisis initiated by seemingly minor irritations is based on a profound systemic reaction, more apparent in those individuals who have an unstable autonomic status.

The balance that is found to exist between the vessels of the splanchnic region and the periphery is naturally one indication of the constantly fluctuating difference in the metabolic activities in the various organs and organ groups.

Minnesota Section.

University of Minnesota Medical School, February 26, 1930.

4847

A Wedge-Photometer for Quantitative Comparison of Ultramicroscopic Particles.

ARTHUR D. HIRSCHFELDER AND HAROLD N. WRIGHT.
From the Department of Pharmacology, University of Minnesota.

In the course of our investigations of the effects of antiseptics and neoarsphenamine on egg albumin and on the colloids of the plasma, it became evident that although the substances tested altered the appearance of egg albumin solutions from the bubble-like picture of lyophillic colloids to the star- and comet-like appearance given by the lyophobic colloids, no such intense change was observable when these drugs were added to oxalated rabbit's plasma, or injected intravenously. It did appear, however, that the bubble-like particles of the plasma became definitely brighter in the presence of the drug. In order to obtain a method for objective recording of this fact, we have devised a wedge-photometer, which is applicable to a variety of investigations of colloidal phenomena.

The hollow wedge-shaped chamber of an ordinary wedge-colorimeter is removed from the colorimeter, the opening at the top is sealed with plastein into which a rubber stopper is fitted, and the chamber is filled with a dilute suspension of a colloid. For this purpose we have found a diluted non-water-proof American India Ink very satisfactory. The wedge chamber which we used was from a home-made colorimeter 30 cm. long, 2.5 cm. wide and 2.5 cm. thick at its thickest portion.

In observing the ultramicroscopic particles the photometer wedge was slid across the ocular of the ultramicroscope until the particles being studied just became invisible. The particles were observed at the instant of their maximum brightness. The distances read off on the wedge were then recorded, and the observation was repeated with the second colloid with which the particles first observed were being

compared. The illumination (small automatic feed carbon arc), magnification, etc., were kept constant. To exclude the subjective factor, the observations were always checked by 2 observers, whose readings coincided within 0.5 cm.

The amount of light reflected is indicated by the thickness of the wedge of opaque fluid necessary to obliterate the particle from view, *i. e.*, by the readings on the wedge, in centimeters.

The apparatus described enables us to compare the appearance of a series of lyophillic colloids with one another under standardized conditions, and should be useful for a considerable number of investigations, particularly for investigation of the degree of hydration of the particles.

We are using it in the investigation of the effects of antiseptics and of various drugs upon blood plasma. Investigations of the blood plasma in various pathological conditions, and of the effects of hydrating and dehydrating agents upon protein and lipoid suspensions, are in progress.

4848

Effects of Neoarsphenamine and of Mercurochrome Upon the Ultramicroscopic Appearance of the Blood Plasma.*

ARTHUR D. HIRSCHFELDER AND HAROLD N. WRIGHT.

From the Department of Pharmacology, University of Minnesota.

In a previous communication[1] we have shown that the addition of a great variety of antiseptics to solutions of purified egg albumin *in vitro* causes the appearance of the albumin particles to change from the bubble-like picture given by egg albumin and other lyophillic colloids, to the punctate, star-shaped and comet-like picture given by lyophobic colloids. We have assumed that this may be regarded as evidence of aggregation and probably also of dehydration.

The conditions observed with blood plasma are, however, somewhat different. The proteins of plasma are probably not like the particles of egg albumin in sodium chloride, naked protein, but certainly represent a better state of dispersion, which is probably in part due to the presence of the plasma lipoids. Under the ultra-

* A part of the funds used in this research were supplied from the Medical Research Fund granted by the Board of Regents of the University of Minnesota.

[1] Wright, H. N., and Hirschfelder, A. D., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 790.

microscope the visible particles never appear as large or as brilliant as those seen in an egg albumin solution, but are smaller and less brilliant.

When neoarsphenamine or mercurochrome solutions are added to oxalated rabbit's plasma (2 mgm. oxalate per cc. of whole rabbit's blood) the spherical particles become definitely more brilliant than they were before. This seems to correspond qualitatively, but not quantitatively, with the actions of these drugs upon egg albumin solution, *i. e.*, there is probably an alteration of the colloid with a partial dehydration and perhaps a small degree of aggregation. When mercurochrome (10 mgm. per kilo) is injected intravenously in a rabbit, and the blood removed 5 minutes or even an hour and a half later, the particles seen when the oxalated plasma is viewed with the ultra-microscope, show the same increase in brilliancy that we observed when the drug was added *in vitro*. Using the wedge photometer[2] for comparing the plasma of the normal rabbit with that of the rabbit injected with mercurochrome, we have obtained the following readings, which are typical for several experiments:

Normal plasma	Rabbit injected with 10 mgm. mercurochrome per kilo, intravenously
4.5—5.0 cm	6.5—7.0 cm.

In harmony with these results we have found that mercurochrome, when injected intravenously, is definitely fixed by, and probably adsorbed on the blood colloids. Though the plasma becomes a deep pink, the mercurochrome will not pass through a viscose sausage skin dialyzer as does an aqueous solution of mercurochrome. An aqueous solution of mercurochrome will stain yeast cells pink; the pink plasma obtained after injection of mercurochrome will not stain yeasts at all.

We are, therefore, forced to the conclusion that mercurochrome, when injected into the veins, converts the plasma colloids, probably the proteins, into aggregates which are foreign to the animal. Since intravenous mercurochrome gives rise to chills and fever similar to that resulting from the injection of foreign proteins, we believe that these new aggregates formed within the patient's own vessels by the intravenous injection of protein act much as though they were foreign proteins; though the reactions are probably not absolutely identical. We believe that it is this foreign protein-like action, rather than an antiseptic action of a drug in concentrations too weak

[2] Hirschfelder, A. D., and Wright, H. N., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 547.

to be either bactericidal or bacteriostatic, which is responsible for any therapeutic effect which may occur in the blood and tissues after the injection of mercurochrome. (Conditions after excretion through the kidney are different, for the mercurochrome is then freed from the protein.)

Since the injection of neoarsphenamine is not so commonly followed by febrile reactions as is that of mercurochrome, it might be expected that the changes which it would produce in the plasma colloids would also be less marked. This is exactly what we have observed with the specimens of neoarsphenamine thus far tested.

After the intravenous injection of 20 mgm. neoarsphenamine per kilo into the rabbit, the plasma particles seem to become definitely more brilliant, but the change is much less marked than that observed after mercurochrome.

We are, therefore, investigating the relation of colloidoclastic reactions observed after the intravenous injection of drugs and in pathological conditions, upon the ultramicroscopic appearance of plasma proteins.

4849

Effects of the Commonly Used Anticoagulants on the Ultramicroscopic Appearance of Frog's Plasma.

HAROLD N. WRIGHT AND RAYMOND N. BIETER.
(Introduced by Arthur D. Hirschfelder.)

From the Department of Pharmacology, University of Minnesota.

A series of investigations are in progress in this laboratory dealing with the changes brought about in the blood plasma by the injection of drugs and also by various pathological conditions.

Since some method of preventing the coagulation of the blood plasma has to be employed, it became essential to study the effects of the commonly used anticoagulants on the appearance of plasma under the ultramicroscope. In previous communications[1, 2] oxalated rabbit's plasma had been used.

Samples of frog's blood *(Rana catasbiana)* were collected into oiled tubes without the use of any anticoagulant, centrifuged, the

[1] Wright, H. N., and Hirschfelder, A. D., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 790.

[2] Hirschfelder, A. D., and Wright, H. N., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 547, 548.

plasma pipetted off and diluted 1:5 with specially prepared, particle free, 0.75% physiological saline solution, and examined under the ultramicroscope. Similar samples were received into heparin (1/3 mgm. per cc.), potassium oxalate (2 mgm. per cc.) and sodium citrate (2.5 mgm. per cc.). The blood was also made non-coagulable by injection of heparin into the dorsal lymph sac 45 minutes previous to bleeding. The appearance of the various samples of plasma was then compared under the ultramicroscope with the normal plasma obtained without the use of any anticoagulant. Quantitative readings were also made of the relative refractiveness of the various plasmas, employing the photometer described by Hirschfelder and Wright.[3]

The use of heparin either *in vivo* or *in vitro* produced only small changes in the appearance of the plasma. With potassium oxalate the particles appeared smaller, greater in number, and of increased refractiveness. Sodium citrate brought about a very marked reduction in the apparent size of the particles, with corresponding increase in the number of particles visible, and increase in refractiveness. This change in the colloidal equilibrium of the plasma proteins with addition of sodium citrate may be of importance in connection with the use of sodium citrate as an anticoagulant in blood transfusions.

Similar experiments on mammalian and human plasma are in progress and will be reported at a later date.

4850

Effect of Novasurol Upon the Ultramicroscopic Appearance of Frog's Plasma.

RAYMOND N. BIETER AND HAROLD N. WRIGHT.
(Introduced by Arthur D. Hirschfelder.)

From the Department of Pharmacology, University of Minnesota.

In a previous paper it has been shown by Bieter[1] that dilute solutions of mercuric chloride injected via the ureter in frog's kidneys, inhibit the reabsorption of water and phenolsulphonephthalein. Unpublished observations in this laboratory indicate that this action might also be shared by novasurol.

[3] Hirschfelder, A. D., and Wright, H. N., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 547.

[1] Bieter, Raymond N., *Am. J. Physiol.*, 1930, in press.

Attempting to obtain further information upon the fundamental action of novasurol as a diuretic, the effects of this drug *in vitro* and *in vivo* have been studied on the appearance of frog's plasma under the ultramicroscope. The technique of the ultramicroscope studies used has been that of Wright and Hirschfelder.[2] The novasurol in 0.1% concentration was made up in 0.75% NaCl. This gives 0.01 mgm. per 0.01 cc. To 1 cc. of frog's plasma diluted with 4 cc. of 0.75% NaCl solution was then added 0.04 mgm. of novasurol.

This amount of novasurol when added to a sample of frog's plasma, which was collected without the use of an anticoagulant, produced the following changes in particles as compared to those described in the preceding paper by Wright and Bieter.[3] The number of particles was decreased, their average size was increased, and their refractiveness, as measured with the photometer of Hirschfelder and Wright,[4] was increased.

When the same amount of novasurol was added to a sample of frog's plasma prepared as above, but where coagulation was prevented by the use of heparin (10 mgm. via lymph sac or 1 mgm. in 0.1 cc. saline placed in the tube used to collect the blood) the picture observed under the ultramicroscope was essentially the same.

The action of novasurol on citrated and oxalated plasmas has again progressed in the same general direction. That is to say, the number of particles was decreased, their average size was increased and their refractiveness as measured with the photometer, was increased. The differences, however, between citrated or oxalated plasmas alone, as compared with the same to which novasurol was added (as above) was not as marked as between heparinized plasma and heparinized plasma plus novasurol.

It is interesting to note further that these effects come on slowly, requiring from 3 to 6 hours for the full effect of the novasurol. This time element compares favorably with the time required clinically for the optimum action of the drug.

Similar experiments on mammalian and human plasmas are in progress and will be reported at a later date.

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[2] Wright, H. N., and Hirschfelder, A. D., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 790.

[3] Wright, H. N., and Bieter, R. N., *Ibid.*, 1930, xxvii, 550.

[4] Hirschfelder, A. D., and Wright, H. N., *Ibid.*, 1930, xxvii, 547.

4851

Monovalent and Polyvalent Antigens for Use in the Diagnosis of Bang's Disease.*

C. P. FITCH, C. R. DONHAM AND LUCILLE BISHOP.

From the University Farm, St. Paul, Minnesota.

The agglutinability of 54 strains of *Bact. abortus* prepared as monovalent antigens and one polyvalent antigen has been studied. Bovine sera showing a large variation in agglutinin content have been employed.

In these studies there was no significant variation in the agglutinability of the monovalent antigens. Any of the cultures would have been suitable for the production of antigen for use in the diagnosis of Bang's disease. There was no advantage or disadvantage in favor of any of the monovalent antigens as compared with the polyvalent antigen. The one polyvalent antigen used was prepared from 8 strains of *Bact. abortus,* all of which were isolated from cattle. The 54 cultures varied in age of cultivation on artificial media from 13 years to a few days. Thirty-nine were isolated at the Minnesota Experiment Station. Four originated in Pennsylvania. Four were received from Connecticut, 3 from New Zealand, one (No. 80) was isolated in California, and one more was received from California that had been isolated in Oregon but cultivated in California for several years, one from New York, and one from Oregon. Of the 39 isolated at Minnesota, 32 were from cattle, 5 from horses, and 2 from swine. Those from cattle were isolated from whole milk, colostrum, placenta, vaginal discharges, foetal lung, foetal stomach contents and from enlarged joints. In one case 4 cultures were isolated from the same animal but from different tissues or fluids. One of the cultures isolated from a bovine was classified by Huddleson as *Para-abortus.*

In preliminary studies, sera from 97 cattle were used, 14 of which showed high agglutinin content, 15 showed medium agglutinin content, 24 had a very low titre, and 44 negative sera with no agglutinin content. In the later studies all of the antigens were tested with the sera of 19 cattle. Of these, one showed a high titre (above 1:5000), one had a titre of 1:1000, fifteen showed low to medium titre (1:25 to 1:250) and 2 were negative. The titre of all of these sera remained fairly constant throughout the course of the experiment with the exception of the serum that was highest in

* This study was aided by a grant from the U. S. Bureau of Animal Industry.

agglutinin content. This one showed a steady decline in its maximum titre.

Technic: In these studies all antigens were made from 4-day cultures that were grown in a 10% CO_2 atmosphere on horse serum agar standardized to pH 7.2 and washed in sterile physiological saline solution containing 0.5% phenol. All antigens were standardized to the same concentration by use of the centrifuge and nepholometer. All tests were set up within 48 hours after the antigens were prepared. Groups of 10 monovalent antigens with the stock polyvalent antigen were set up at one time. Fresh sera were always used. Observations were made at 24, 48, 72, 96, and 120 hour intervals. The tests were incubated at 37.5° C.

Results: With High Agglutinin Content Sera (Titre above 1:1000). There was considerable variation in the maximum agglutinability of the monovalent antigens, *e. g.,* occasionally a serum would agglutinate one antigen up to 1:10,000 and another antigen only 1:4000. In no case was an antigen found which failed to be agglutinated in 1:1000 dilution with high titre serum. There was no apparent relationship between speed of agglutination and maximum agglutinability of the monovalent antigens. That is, a slow agglutinating antigen often showed a higher titre after 72 to 96 hours than another antigen which agglutinated more rapidly in the lower dilutions. The maximum titre was usually not reached until the 96 hour reading, but there was practically no change between the 96 and 120 hour reading.

With Low to Medium Agglutinin Content Sera (Titre 1:25 *to* 1:500). No significant variations in agglutinability of the antigens with this group of sera were encountered. The slight variations that were recorded were not consistent and were within the limits of error of technic and observation. In no case would the diagnosis have been changed by these variations. The maximum titre was usually reached at the end of 48 hours but occasionally a slightly higher titre was observed at the 72 hour reading.

With Negative Sera. None of the antigens used in these studies showed agglutination with negative sera except in the preliminary studies where the sera from 76 supposedly negative cattle were used. In this group there were 26 sera which did show some agglutination in the 1:25 and 1:50 dilutions. Such sera were not selective but showed agglutination in these dilutions with practically all of the antigens. The slight variations were not consistent and were within the limits of error. Agglutination in these cases may or may not have been due to specific agglutinins in the sera.

Other Observations. Cattle tested with monovalent antigens, prepared from cultures that were isolated from their own tissues, did not show any difference in titre than when other antigens were used. This has been noted with 3 animals. Three monovalent antigens became contaminated with other bacteria. At this time they gave a much higher titre with all types of sera than other pure antigens. When antigens were prepared from cultures of these strains that were not contaminated, this discrepancy was not in evidence.

Western New York Section.

University of Buffalo Medical School, February 15, 1930.

4852

Passage of Water Through Frog Skin in Relation to Temperature.

EDWARD F. ADOLPH.

From the Physiological Laboratory, The University of Rochester School of Medicine and Dentistry.

The rate of passage of fluid through the skin of an intact frog is markedly greater with increase of temperature, when frogs with cloacae ligated are transferred to any NaCl solution. In a hypotonic solution the frogs gain weight and therefore water; in a hypertonic solution they lose water. If desiccated and then placed in water they restore fluid to the body faster with increase of temperature, but the relative effect of temperature is smaller. All these influences might be due to changes in the rate of the circulation rather than to changes in the skin. If isolated skin is placed as an osmometer membrane between 2 solutions, a large increase in rate of passage with increase of temperature is manifested. But at higher temperatures the passage may be reversed in direction in certain solutions, rather than augmented in rate in the original direction.

There is no doubt that temperature influences the rate of passage through the skin independently of other tissues; but there is no constant temperature coefficient which holds over a large range. It is known that even isolated skin has more than one differentiable factor involved in its control of the passage of water. Returning to the intact frogs, the influences of temperature upon those forces that are present when frogs are placed in hypotonic solutions may be measured separately from the influences upon the force of osmotic pressure which is present in and proportional to all strengths of solutions. It is found that the movement of water due to all the forces increases very largely with temperature; the movement due to osmotic pressure gives coefficients from 1.4 to 5.0 for 10° increase of temperature, which values of the coefficient are, in each experiment, about as large as the coefficient of the movement due to other forces.

4853

The Influence of Yeast on Nitrogen Retention in Normal and Depancreatized Dogs.

E. S. NASSET AND H. B. PIERCE. (Introduced by J. R. Murlin.)

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

One normal dog and 4 completely depancreatized dogs were fed a basal diet with additions of baker's and starch-free yeast in 4 to 6 day periods. The control dog showed a greater nitrogen retention in the yeast periods than in the control periods, amounting, in the first weeks of the experiment, to 150% of the extra nitrogen ingested in the yeast; at the end of 3 months this had decreased to 30%. Following a control period of 3 months the nitrogen retention on a subsequent yeast regime was 190% of the nitrogen contained in the yeast. The high retention was again noted after a month during which the dog had been on stock diet. The loss of nitrogen in the depancreatized dogs was less in the yeast periods than in the control periods, with no significant alteration in the D:N. There was no apparent correlation between the distribution of waste nitrogen to urine and feces and the addition of yeast to the diet.

4854

Can the Isolated, Perfused Liver of the Dog Form Carbohydrate at the Expense of Fat?

DONALD E. GREGG. (Introduced by J. R. Murlin.)

From the Department of Vital Economics, University of Rochester, Rochester, N. Y.

Eighteen perfusions, 4 using cats and 14 using dogs, have yielded essentially negative results. These animals were fed on XXXX cream for periods extending up to 30 days. The livers were placed on a weighing scale in an incubator kept at 37.5° C. Thirteen perfusions were single, that is, blood was perfused through the portal vein only. Five were double, that is, blood was perfused both through the portal vein and the hepatic artery.

The exclusive fat diet did not remove all the glycogen from such livers, many of which showed a normal glycogen content. The percentages of glycogen and free sugar of the various lobes in each liver showed marked differences.

In such perfusion systems the true blood sugar, blood non-fermentable reducing substance, urea and ammonia nitrogen and lactic acid all increased. Both the liver glycogen and free sugar showed substantially lower values at the close of the perfusions. The blood fatty acids were constant.

In one experiment the total carbohydrate of the perfusion system increased; in 4 a decrease occurred; the rest were unaltered. The 5 double perfusions showed a marked constancy of the total carbohydrate content, all values at the close of the perfusion being within 7% of the initial value. The total fatty acids were fairly constant, 2 experiments showing an increase and 5 a reduction.

The data obtained do not substantiate the hypothesis that carbohydrate is produced at the expense of fat.

4855

Absorption of Epinephrine from the Subcutaneous Tissue of the Rat.

C. F. CORI AND G. T. CORI.

From the State Institute for the Study of Malignant Disease, Buffalo.

In a previous investigation of the carbohydrate metabolism of rats a dose of 0.2 mg. of epinephrine per kilo was injected subcutaneously.[1] This made it desirable to find out whether such a dose produced a rise in blood pressure. The animals received amytal intraperitoneally, followed by the injection of a small amount of urethane in order to steady the blood pressure. A glass cannula was tied into the carotid artery and connected with a mercury manometer of small dimensions. Heparin was used as anticoagulant in the cannula. Intravenous injections were made into a femoral vein and were timed by the beats of a metronome. In 4 experiments of the type shown in Fig. 1 the minimal pressoric rate of intravenously injected epinephrine was established before the subcutaneous injection was made. It was found that an intravenous injection of 0.001 mg. per kilo per minute was always followed by a rise in blood pressure, while a subcutaneous injection of 0.2 mg. per kilo made shortly afterwards had no effect on blood pressure. This result permits the conclusion that the absorption of epinephrine from the subcutaneous tissue of the rat proceeds at a rate less than 0.001 mg.

[1] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1928, lxxix, 309, 321, 343.

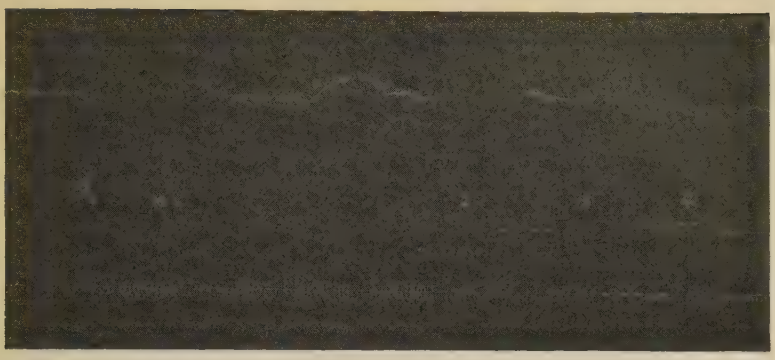

FIG. 1.

Male rat under amytal-urethane anesthesia. Weight 200 gm. Time in 5 seconds at base line of mercury manometer.

(0) 0.25 cc. of 1 to 1,250,000 injected in 1 minute or 0.001 mg. epinephrine per kilo per minute.

(1) 0.25 cc. of 1 to 835,000 injected in 1 minute or 0.0015 mg. epinephrine per kilo per minute.

(2) 0.25 cc. of 1 to 625,000 injected in 1 minute or 0.002 mg. epinephrine per kilo per minute.

(3) 0.04 mg. epinephrine injected subcutaneously.

(4) 0.25 cc. salt solution injected in 1 minute.

per kilo per minute. How much less the rate is can of course not be judged from these experiments. Since a subcutaneous injection leads to hyperglycemia and since an intravenous injection of 0.0002 mg. per kilo per minute is the smallest amount which still produces hyperglycemia in rats, the rate of absorption of epinephrine from the subcutaneous tissue must be above this figure.

In order to ascertain how long epinephrine remains in the subcutaneous tissue, chemical determinations were carried out. Rats of 250 to 300 gm. of body weight were anesthetized with amytal. A subcutaneous injection of 0.1 mg. of epinephrine was made on the dorsal side of either the hind or foreleg. At various time intervals after the injection the "depot" of epinephrine, often plainly visible in the loose connective tissue under the skin, was excised. Bleeding was avoided. The whole region of the injection was rinsed with water. After grinding and extracting the small bits of tissue in a mortar and removing traces of protein by means of the Folin-Wu precipitation, the epinephrine content was determined by means of the Folin-Cannon-Denis method.[2] Great accuracy cannot be claimed, because only 80% of the amount injected could be recovered from the subcutaneous tissue of dead animals immediately after the injection. This immediate loss may be due partly to oxidation upon exposure to air, because the injected fluid in the subcutaneous tissue

[2] Folin, O., Cannon, W. B., and Denis, W., *J. Biol. Chem.*, 1912, xiii, 477.

assumes a purplish tinge when the skin is cut. A considerable amount of epinephrine is also lost when it remains for some time under the skin of dead animals, as is shown in Table I. This indi-

TABLE I.

Recovery of epinephrine from subcutaneous tissue of rat.

0.1 mg. was injected in each case. The values are expressed in % of the amount injected.

	Immediately	Ave.	After 1 hour	Ave.	After 2 hours	Ave.	After 3 hours	Ave.
Dead rats	79; 83; 80; 78	80	65; 60; 61	62	38; 45; 40	41		
Live rats	78; 84; 81; 80	81	47; 51; 43; 58	50	32; 49; 20; 25	32	17; 18; 9; 16; 11	14

cates that the disappearance of epinephrine from the subcutaneous tissue of live animals depends not only on absorption into the blood stream but also on local destruction. The data of Table I can therefore not be used for a calculation of the rate of absorption of epinephrine into the blood stream. It is concluded from the experiments in Table I that epinephrine is still present in the subcutaneous tissue 3 hours after the injection and this direct chemical evidence agrees well with the observation that the hyperglycemia persists for at least 3 hours.

4856

The Influence of Constant Intravenous Injection of Epinephrine on Blood Sugar of Rats.

C. F. CORI AND G. T. CORI.

From the State Institute for the Study of Malignant Disease, Buffalo.

The aim of the present experiments was a determination of the minimal rate of injection of epinephrine which causes a rise in blood sugar of rats. Similar experiments on rabbits could be made without anesthesia but this was unfortunately not possible on rats. Though amytal anesthesia has little effect on the blood sugar of rats, as may be seen in Table I, it undoubtedly lowers the carbohydrate tolerance. Animals in the postabsorptive state with a high liver glycogen content on account of previous carbohydrate feeding were used. 0.1 to 0.2 cc. of blood was withdrawn from a femoral vein by means of a syringe at the beginning and end of the injection. A

third sample was taken 30 minutes after the end of the injection. The temperature of the animals was kept between 97.8 and 99° F., heat being supplied by an electric bulb. The dilutions of epinephrine were made with a slightly acidified physiological salt solution and as a control the same salt solution was injected. The injections of epinephrine lasted for 30 minutes. It is concluded from the data of Table I that 0.0002 mg. per kilo per minute is the smallest rate of injection which produces a definite rise in blood sugar of amytalized rats.

TABLE I.

Influence of epinephrine on blood sugar of rats under amytal anesthesia.

Weight	cc. injected in 30 min.	mg. epinephrine per kilo per minute	Blood sugar (in mg. per 100 cc.)		
			Before injection	After injection	30 min. later
176	1.46	0.001	116	171	155
170	1.43	0.001	122	178	141
173	1.21	0.0005	128	192	
206	1.50	0.0004	108	155	121
205	1.56	0.0004	119	173	123
220	1.88	0.0003	108	163	
203	0.96	0.00025	111	134	119
188	1.59	0.0002	117	128	110
184	1.13	0.0002	114	147	124
194	0.63	0.00015	122	109	114
200	1.14	0.0001	114	115	
223	1.54	0.0001	112	114	104
212	1.50	salt solution	113	105	109

TABLE II.

Response of different species to constant intravenous injection of epinephrine.

Species	Minimal pressoric rate	Minimal hyperglycemic rate
	mg. epinephrine per kilo per minute	
Man	0.00005	0.000025
Rabbit*	0.0006	0.00005
Rat†	0.001	0.0002

* Unanesthetized. † Under amytal anesthesia.

The relation between the pressoric and hyperglycemic rate of epinephrine injection in different species, as determined recently in this laboratory, is shown in Table II. This comparison shows that the carbohydrate metabolism and the vascular system of the rat is decidedly less sensitive to epinephrine than that of the other species investigated.

New York Meeting.

New York Academy of Medicine, March 19, 1930.

4857

A Carbohydrate Isolated from Monilia Psilosis.

D. H. COOK, H. D. KESTEN AND J. W. JOBLING.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City, and the School of Tropical Medicine of the University of Porto Rico, under the auspices of Columbia University.

In the light of the recent work on specific carbohydrates of various organisms, it seemed advisable to test the nature of one of the *Monilia* group of fungi in this respect. *Monilia psilosis,* Ashford, was chosen because of its possible relation to tropical sprue. Kurotchkin and Chu,[1] using a *Monilia tropicalis,* isolated from a case of bronchomoniliasis, obtained complement fixation and weak precipitin tests with an alkaline extract of the organism and with the alcoholic precipitate from this extract. No further attempts at purification of this material were reported.

The method of extraction, as first used, was that of Salkowski as given by Mueller and Tomcsik[2] in their work on yeast gum. Six day cultures of *Monilia psilosis* grown on honey broth were centrifuged, washed with water followed by alcohol, and extracted over-night twice with ether. The organisms, when treated by the method of Mueller and Tomcsik, gave an active material as indicated by the precipitin reaction. The method was later modified in that the monilia bodies were given (after alcohol washing and ether extraction) a preliminary treatment with Fehling's solution, 100 cc. for every 10 gm. of the dry material. The violet-colored material was centrifuged off and washed 3 times with distilled water, thus removing considerable protein dissolved by the alkali. The colored residue was then decolorized with N HCl and centrifuged or fil-

[1] Kurotchkin, T. J., and Chu, C. K.; *Nat. Med. J. China,* 1929, xv, 403.

[2] Mueller, J. H., and Tomcsik, J., *J. Exp. Med.,* 1924, xl, 343.

tered. The clear liquid contained the active material. This liquid was treated with Fehling's solution, which caused the separation of a blue, flocculent precipitate, the active material. This, after two washings with distilled water by centrifugation, was dissolved in 10 cc. of N HCl for each 10 gm. of the original monilia. If the solution was not entirely clear, 95% alcohol was added to the point of first increased opacity, generally about one volume. Prolonged centrifugation threw down impurities, leaving a water-clear solution. From here on the technique was that used by Mueller and Tomcsik, consisting of repeated precipitation with 3 or 4 volumes of alcohol from dilute acid solution, followed by washing and drying with ether.

The final product is a white, friable material, which gelatinizes and slowly dissolves in water. It gives a Molisch test in a dilution of 1:1,000,000. The best sample contains 0.58% N,* and though it gives a faint bluish-red biuret in 2% solution on standing several minutes, the Hopkins-Cole, xanthoproteic, Robert's, trichloracetic, Millon, and ninhydrin tests are all negative. Where possible, the tests were carried out upon the solid material to increase sensitivity. It is precipitated by basic lead acetate or glacial acetic acid, but not by neutral lead acetate. From these results it would appear that the active material is non-protein in nature, though the presence of the nitrogen makes it impossible to state this with finality. Sufficient material has not been available to do a Van Slyke amino-nitrogen determination.

The carbohydrate gives no reduction on boiling with Fehling's solution, but after 15 minutes' hydrolysis with N HCl at 100°, reducing sugars are present. Glucosazone was prepared from the hydrolysate and identified by its crystalline form. Glycogen is absent by the iodine test. The naphtholresorcinol test gives a reddish-brown ether solution showing two absorption bands, one in the red, the other in the green, thus indicating the possibility of a uronic acid linkage. Lack of material has hindered more complete investigation of the chemical nature of the carbohydrate. Calculated on the dry weight of monilia, the yield of purified carbohydrate is about 1%.

Using an antiserum prepared in rabbits by the injection of a suspension of killed *Monilia psilosis,* and having an agglutination titer of 1:400, precipitin tests were done on various preparations of the carbohydrate. Precipitin rings were regularly obtained in dilution of 1:100,000 in ½ hour and often within five minutes. Occasionally a ring was formed in one hour at a dilution of 1:1,000,000. Nor-

* Dumas micro-nitrogen determinations were kindly done in the laboratory of Professor J. B. Niederl, Washington Square College, New York University.

mal rabbit serum and anti-sera prepared against a strain of *Willia* and against Fleischmann's yeast were negative at 1:100 dilution of the polysaccharide. The activity of a 1:100,000 solution was apparently undiminished after heating 30 minutes at 100° C.

In view of the specificity of most of the other polysaccharides isolated by various workers it is of interest to note that precipitin rings were obtained with *Monilia psilosis* polysaccharide using anti-sera† prepared against similar yeast-like organisms, but either at a lower dilution or less rapidly, in the majority of cases. (These included *Monilia albicans, Monilia parapsilosis,* Ashford, *M. krusei,* Castellani, a non-pathogenic monilia, and two strains of monilia isolated from erosio interdigitalis.)‡ This raises a possible question as to the individuality of the species so far tested and is further confirmation of the findings of Hopkins and Benham,[3] who observed cross-agglutinations among these same organisms, although certain distinctions could be obtained by agglutinin absorption. Work is in progress to obtain the carbohydrates from related species with the object of studying cross-precipitin reactions and precipitin absorption.

With the isolation of additional active material it is hoped to amplify both the chemical and immunological studies including skin and serum reactions of individuals harboring monilia.

4858

The Dietary Production of Dystrophy of the Voluntary Muscles.*

MARIANNE GOETTSCH. (Introduced by A. M. Pappenheimer.)

From the Laboratory of Biological Chemistry, College of Physicians and Surgeons, Columbia University of New York.

Vitamin E has been shown by Evans, Bishop, and their coworkers[1, 2, 3] to be necessary for normal reproduction in the rat, and by

† Kindly furnished by Miss R. W. Benham, Department of Dermatology, College of Physicians and Surgeons, Columbia University.

‡ It is interesting that the monilia polysaccharide precipitated with a sample of Type II pneumococcus antiserum furnished by Dr. M. Heidelberger.

[3] Hopkins, J. G., and Benham, Rhoda W., *N. Y. State J. Med.,* 1929, xxix, 793.

* This work has been conducted with the aid of the departmental Research Fund of the Chemical Foundation.

[1] Evans, H. M., and Bishop, K. S., *J. Met. Research,* 1923, iii, 201, 223.

[2] Evans, H. M., and Burr, G. O., *Memoirs of U. of Calif.,* Berkeley, 1927, viii.

[3] Evans, H. M., and Burr, G. O., *J. Biol. Chem.,* 1928, lxxvi, 273.

Beard,[4] in the mouse. This investigation was begun in order to study the effect of an E-free diet on the guinea pig; and as the problem of feeding these animals simplified diets, such as are ordinarily used in vitamin E experiments, was rather a difficult one, it was decided to use instead, a natural food diet, in which the vitamin E had been destroyed by treatment with ethereal ferric chloride, a method discovered by Waddell and Steenbock.[5] When guinea pigs were reared on this diet, they stopped growing after 1 to 2 months, maintained a constant weight for another month, rapidly declined for 2 or 3 days, and suddenly died. The oestrus rhythm was found to be normal but further studies upon reproduction were impossible. Upon autopsy, these animals showed marked macroscopic changes in the skeletal muscles throughout the body.

The diet was prepared as follows:

Rolled Oats (Quaker)	355 parts
Wheat Bran (Pillsbury)	180 "
Casein (Merck Technical)	75 "
Lard	80 "
Cod Liver Oil (Meads)	10 "
NaCl	10 "
$CaCO_3$	15 "

The ingredients were shaken in a closed container with 10 gm. of ferric chloride, U.S.P. lump, that had been taken up in about 125 cc. of ether and a little water, and the mixture set aside. After half an hour, the contents were transferred to a tray and the ether allowed to evaporate. Then there was added:

Skimmed Milk Powder (Merrell Soule) 275 parts.

Each guinea pig was given daily by pipette 3 cc. of orange juice, to protect it from scurvy.

Rats, both male and female, reared upon this diet, gave symptoms that were typical of vitamin E deprivation; and the females were cured after true resorption of the embryos by transferring them to a diet, similar in all respects except that the ethereal ferric chloride treatment had been eliminated.

Guinea pigs that had been born in the laboratory were used for these experiments, and upon weaning at 21 days, they were given the experimental ration with greens and orange juice. After about 10 days, when they had become more or less accustomed to the new diet, the greens were discontinued. Control guinea pigs were given

[4] Beard, H. H., *Am. J. Physiol.*, 1926, lxxv, 682.

[5] Waddell, J., and Steenbock, H., *J. Biol. Chem.*, 1928, lxxx, 431.

either a similar diet in which the ethereal ferric chloride treatment had been omitted or else the stock diet of oats, bran, hay and greens.

Thus far 20 animals on the experimental diet have been studied and 10 controls. The experimental group had rather similar histories, which differed markedly from litter-mate controls. The growth rate for the first month or two was normal. After the animals had attained a weight of about 400 gm., there was a sudden cessation in the growth of the experimental group accompanied by general flabbiness; and it was observed that the animals had difficulty in righting themselves if they were placed on their backs, a test used by the California workers[3] to determine the degree of paralysis in baby rats. During the following month, a constant weight was maintained after which the animals went into rapid decline for 2 to 3 days and suddenly died on about the hundredth day of the experiment, although one animal is still living after 7 months on the diet. The controls showed no break in the growth curve and continued to grow steadily. A few of the experimental group recovered spontaneously from the flabbiness, made a moderate gain in weight, remained stationary at a higher level, and died.

While most of the animals were killed and autopsied when apparently on the point of death, a few were sacrificed before the cessation of growth. Of the 19 experimental guinea pigs that have come to autopsy, all but one showed striking macroscopic changes in the voluntary muscles. The muscles of the thigh and abdomen were particularly involved. They appeared atrophied and pale, and had a yellowish color, quite different from those of the normal controls. Sometimes they were gritty looking, and streaked as though calcified or infiltrated with fat. In 2 cases, the thigh muscles were markedly hemorrhagic. The muscles seemed to have lost their irritability.

The other organs appeared normal. In no case were there any scorbutic lesions. The animals were thin and flabby, but not particularly emaciated, as indicated by normal deposits of fat in the subcutaneous tissue. Inanition was not the cause of the muscle dystrophy because some of the most pronounced lesions were found in actively growing animals that showed no decline in weight.

Rats reared on this diet seemed normal in all respects except for the sterility caused by the absence of vitamin E from the diet.

Two rabbits born in the laboratory were given the same diet with 6 cc. of orange juice when they were 4½ weeks of age. They maintained their weight and even grew to some extent for 12 days, then lost the use of their muscles completely, and were killed and autop-

sied 3 and 4 days later. The muscles were small, extremely pale, and without irritability. Other organs seemed normal.

Experiments are in progress in which guinea pigs are to be killed at different stages in order to determine the time of onset of the disease. Curative, as well as prophylactic doses of wheat germ oil are being administered to guinea pigs and rabbits in order to ascertain whether vitamin E deficiency is the cause, or whether the toxicity of the ferric chloride itself, or its effect on the natural food diet in some way other than vitamin E destruction may be responsible. The muscles of paralyzed baby rats as produced by Evans and Burr and their coworkers[3] upon vitamin E-free diets are being investigated.

The only conclusion which may be drawn at present is that the diet described in this paper produces in the guinea pig and rabbit a general dystrophy of the voluntary muscles unaccompanied by obvious lesions in the other organs.

4859

Pathological Changes in the Skeletal Muscles Produced by Dietary Means.

ALWIN M. PAPPENHEIMER.

From the Department of Pathology, Columbia University, New York City.

Guinea pigs maintained on the diet described in the previous paper[1] for periods ranging from 35 to 133 days, develop extreme degeneration of the skeletal muscles of the trunk and extremities.

The primary alteration is a waxy or hyaline necrosis of the fibers. This is followed by great proliferation of the muscle nuclei, leading to the formation of so-called "Muskelzellenschlauche" within the intact sarcolemma. There is also active regeneration of muscle cells in the later stages. The disappearance of the degenerated fibers is accompanied by a variable amount of interstitial fibrosis and lipomatosis.

The affected muscles are characterized grossly by a striking pallor.

The prevailing color is yellowish grey, with fine stippling. The muscular tone and elasticity are lost, and the muscle bulk is much reduced in comparison with that of control litter mates.

Careful histological study of the principal organs and tissues other

[1] Goettsch, M., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 564.

than the skeletal muscles shows no significant change. The brain has not been routinely examined, but in sections of the spinal cord, the motor ganglion cells at all levels are normal in appearance. There are also no obvious lesions in the peripheral nerve trunks. The heart muscle, and the smooth muscle of the gastro-intestinal tract, bronchi, blood vessels and uterus are not affected.

Two young rabbits were examined on the 15th and 16th days after having been placed upon the experimental diet. At that time, they were extremely weak, lying flat on their belly, with limbs flaccid and outstretched. The head could not be raised from the table. The muscles were uniformly pale, grey and translucent, not stippled. Microscopically, the lesions were identical with those seen in the guinea pigs. Necrotic fibers were plentiful, but there had already occurred enormous multiplication of muscle nuclei, and active regeneration of new fibers was in progress. No other visceral lesions were detected.

The experimental diet thus brings about a universal dystrophy of the entire voluntary muscular system, and no significant lesions have as yet been found in any other tissue or organ. Inanition and scurvy may be excluded as possible factors.

4860

A Thermal Conductivity Recorder for Oxygen and Carbon Dioxide For Clinical Atmosphere Control.*

GRACE LUBIN AND JESSE G. M. BULLOWA. (Introduced by W. H. Park.)

From the Medical Service of Harlem Hospital, New York City.

Thermal conductivity instruments, which in the past 10 years have been increasingly applied in industry and in medicine, have hitherto not been available for the measurement of oxygen in air, since it was assumed that the thermal conductivity of oxygen was too close to that of nitrogen, and hence to that of air, for the practical application of the method to this type of mixture. Recent studies at Harlem Hospital have indicated, however, that the thermal conductivity instruments used to determine the carbon dioxide content of alveolar air are more sensitive to changes in oxygen concentration than has generally been assumed to be the case. Indeed,

* Research supported by the Committee for the Encouragement of Medical Research through New York University.

the galvanometer deflection caused by a 10% increase in oxygen concentration is about the same as that caused by a 1% decrease in carbon dioxide concentration. This observed effect is held to be consistent with the relation between the accepted thermal conductivity values for oxygen, air and carbon dioxide.

These preliminary experiments showed that a thermal conductivity instrument cannot be used for carbon dioxide measurement in the presence of a varying oxygen concentration, unless the thermal cell is rendered indifferent to these variations in oxygen by passing the gas stream to be tested through both the analyzing and the standard or reference compartments of the cell, the reference mixture being freed from carbon dioxide with a suitable scrubber.† The tests also suggested that the single-flow thermal cell, with normal air in the fixed reference mixture, which is used to measure carbon dioxide in air to within +0.05%, could be directly calibrated to indicate within 0.5% the concentration of oxygen in a stream of air mixture, freed from carbon dioxide.

Prompt and generous cooperation on the part of Chas. Engelhard, Inc., of Newark, N. J., and especially of Dr. W. F. Hamilton, research director, resulted in the construction and calibration of a 2-cell instrument, which yields on a single chart the quasi-continuous record of the concentration of carbon dioxide on the one hand and of oxygen on the other hand. The preliminary results obtained with this instrument on the oxygen and carbon dioxide enriched air of the atmosphere control room at Harlem Hospital have shown satisfactory agreement with independent chemical analyses. It is planned to continue these tests over a considerable period of time, and also to study the applicability of thermal conductivity indicators in connection with the administration of oxygen under a portable tent.

† When carbon dioxide analysers are applied to gas mixtures with a constant CO_2/O_2 ratio, as for flue gas control and under basal metabolism conditions, the disturbing effect of variations in oxygen concentration is practically eliminated by calibration of the instruments under the conditions for which they are to be used.

4861

Observations on Streptococcus Toxin-Antitoxin Neutralization as a Basis for Specificity.

MARY W. WHEELER. (Introduced by A. B. Wadsworth.)

From the Division of Laboratories and Research, New York State Department of Health, Albany.

Practically all our experience with the interaction of the toxins and antitoxins of the diphtheria and tetanus bacilli indicates that this reaction is one of the most specific immune reactions, if not the most specific, upon which to base conclusions concerning the difference of species or of subgroups of species. In the study of the hemolytic streptococci, however, two sharply contrasted points of view concerning the specific relation of the toxins and antitoxins to the various disease processes have developed despite extensive research.

Our studies of the streptococci appear to throw additional light on the nature of the toxin-antitoxin reaction and suggest why such divergent conclusions may be reached in the study of these organisms. I am, therefore, presenting these observations from this point of view.

During the past 6 years the toxicity of about 200 strains of hemolytic streptococci from typical cases of scarlet fever and as many more from cases of erysipelas, septic sore throat, and other streptococcus infections has been determined.

Man and goats are the only animals susceptible to the streptococcus toxins, with the possible exception of the rabbit. Different persons and different goats vary in their susceptibility to the toxins of different strains and an individual person or goat does not always react to the same degree to the same toxin. For example, in tests where 2 "scarlet fever" toxins, A and B, and 4 "erysipelas" toxins, C, D, E, and F, were tested on each of 3 individuals, person No.1 reacted in an equal degree to toxins A, B, D, and E and slightly to toxin F but failed to react to toxin C. Person No. 2 reacted equally to toxins A, B, D, and E but did not react to C or F; while person No. 3 reacted to the 4 erysipelas toxins and to the scarlet fever toxin A but failed to react to the scarlet fever toxin B. Goats susceptible to scarlet fever toxin A in high dilutions were also susceptible to all 4 erysipelas toxins. The scarlet fever toxin B, however, induced reactions only when large doses were given. In another instance 2 toxins of streptococci from cases of epidemic septic sore throat were

tested on each of 2 persons susceptible to the scarlet fever toxin A. Each person reacted to only one of the "septic sore throat" toxins but each to a different one. One of these septic sore throat toxins induced a definite reaction in goats susceptible to toxin A, the other no reaction even in low dilutions.

When repeated tests have been made on the same individual and a period of a month or more has elapsed between tests, variations in susceptibility to the same toxin have been noted. In one person tested repeatedly with 2 scarlet fever toxins, susceptibility to both toxins increased, in 2 others there was a decrease. On the other hand, the reactions induced in 4 persons by one of these toxins became more marked while the reactions induced by the second remained the same. A fifth person became more susceptible to the second toxin and less susceptible to the first.

Although it was apparent that the toxins produced by different strains varied, no significant differences in the toxins of streptococci from different sources have been observed either in potency or in their neutralization by antitoxin. The toxicity of streptococci from all sources varied, some apparently producing no demonstrable toxin; others, toxins of higher or lower potency. Eighty-five per cent of the strains from all sources have produced toxins inducing definite skin reactions in goats.

When varying doses of toxin and serum have been employed, 65 to 70% of all the toxins, irrespective of the source of the strain, have been neutralized by one monovalent antistreptococcus goat serum produced by a representative strain selected as a standard for purposes of comparison. This strain, the Dochez N. Y. 5 strain, was isolated from a typical case of scarlet fever and has been the incitant of 2 typical cases of this disease in members of the laboratory staff. About 35% of scarlet fever toxins and practically the same proportion of toxins of streptococci from other infections have not been neutralized by this serum even when large doses have been employed.

Tests with standardized toxins and antitoxins when only one or 2 skin test doses of toxin have been used and corresponding doses of a monovalent serum, have also given evidence of the similarity of toxins of streptococci from different infections. Under these conditions erysipelas toxins have been neutralized by monovalent scarlet fever antistreptococcus sera, and scarlet fever toxins by monovalent erysipelas antistreptococcus sera.

Differences in the toxins and sera not apparent in the usual toxin-antitoxin neutralization test were brought out when the combining

power of sera for homologous and heterologous toxins was determined. Apparently neutral mixtures of serum and various heterologous toxins were found in some instances to have no neutralizing activity for the homologous toxin; in others, the neutral mixtures possessed an activity only slightly less than that of the same dose of serum alone.

Carefully controlled titration tests of sera against homologous and heterologous toxins have shown marked differences in the neutralizing activity of monovalent sera produced with different streptococci, doubtless corresponding, in some degree at least, to the variations in the action of the toxins which give rise to them. Sera produced by certain strains have been found to be broadly valent and capable of neutralizing toxins from various sources. Other monovalent sera have been found to be of limited valency and to have little demonstrable activity for heterologous toxins, even those of streptococci from the same type of infectious process.

The serum of horses immunized with the Dochez N. Y. 5 strain had an equal neutralizing activity for the homologous toxin and for the toxins of the Dick II strain and for those of certain erysipelas strains, while 2 monovalent goat sera produced against different erysipelas strains neutralized erysipelas toxins and also the N. Y. 5 toxin.

On the other hand, the serum from a horse immunized with the Dick II strain has been found, when tested on some individuals, to have an equal neutralizing activity for both the Dick II and the Dochez N. Y. 5 toxins. When tested on other individuals, however, this serum neutralized in high dilutions its homologous toxin, but had little or no apparent activity for the N. Y. 5 toxin.

Monovalent sera produced in animals of different species with the same strain have also been found to vary in their neutralizing ability for different toxins. Toxins which have not been neutralized by an antistreptococcus goat serum produced by the N. Y. 5 toxin have been neutralized by an antistreptococcus horse serum produced by this same strain.

Animals immunized with other toxic strains of streptococci have shown little or no response even though the immunization has been continued over a long period.

Further evidence that certain strains of streptococcus may be broadly valent in their antigenic activity was apparent from the reactions induced by different toxins in persons before and after immunization with the toxin of one strain.

Children susceptible to both erysipelas and scarlet fever toxins,

after immunization with the scarlet fever toxin, reacted less strongly or not at all to twice the amount of the toxins used in the first test

Summary and Conclusions. Thus from the variations observed in the reactions induced in different persons and different animals of the same species by the various toxins studied and by mixtures of these toxins with different antistreptococcus sera, it would appear that both the streptococcus toxins and antitoxins are quite complex. It would also appear that the action of these toxins in the animal body and their neutralization by antitoxin depend upon particular phases of tissue susceptibility which may be present in one individual and not in another.

Toxins produced by streptococci from various sources appear to be quite similar when tested with a monovalent serum of broad valency. On the other hand, toxins neutralized with such a serum may be found to be quite dissimilar when tested with another serum produced by a different strain or in an animal of a different species or even in different persons or animals. These differences, however, do not appear to be related in any way to the type of infection from which the streptococcus was isolated.

Hence, if toxins of limited activity and antitoxins of narrow range are used and a small number of strains of streptococci are tested, it is quite conceivable that the results would suggest the possibility of dividing the streptococci into apparently specific groups. On the other hand, if representative strains of broad antigenic activity and antitoxins of wide valency are selected and a large number of representative strains from different types of streptococcus infections are studied, it is evident that so many strains would coincide in their reactions, that it is impossible, in the light of our present knowledge, to define the specificity of streptococci in relation to a particular type of infection.

4862

Identity of Animal Anaphylaxis and Human Allergy (Protein Hypersensitiveness).

BRET RATNER AND HELEN LEE GRUEHL.

From the Departments of Immunology and Pediatrics, University and Bellevue Hospital Medical College, New York City.

The assumption that the symptoms of anaphylactic dyspnea in the guinea pig and the symptoms of dyspnea in the asthmatic patient can be explained on the common basis of anaphylactic hypersensitiveness was believed by the earlier investigators. Curiously enough this logical conclusion seems to have created a point of departure which resulted in a large series of studies particularly championed by Coca, Cooke and their co-workers to differentiate anaphylaxis in the animal and hypersensitiveness in the human being. This has succeeded in complicating one of the most important fundamental problems in hypersensitiveness.

In a most recent summation of his differentiation between anaphylaxis in the lower animal and human hypersensitiveness, Coca[1] makes the following statements. Anaphylactic antibodies in the lower animal react with antigen and cause shock; antibodies neutralize antigen and thus produce desensitization; they sensitize smooth muscle but not human skin.

In the differentiation of anaphylaxis and hypersensitiveness Coca found it necessary to devise the new terms "atopy" and "reagin". By atopy Coca would designate certain clinical forms of hypersensitiveness in human beings; these individuals do not acquire their sensitization as do the lower animals, but are born with this particular hypersensitiveness that is inherited. The substance present in the blood of such atopic individuals is not an anaphylactic antibody but a specific body which he terms "skin-sensitizing reagin". Individuals born with true reagins are exquisitely sensitive. Reagins can be passively transferred to a normal human skin but cannot sensitize smooth muscle. There is no phenomenon of desensitization in human hypersensitiveness.

Many attempts have been made to transfer human hypersensitiveness passively into the animal. In a general sense the results obtained were only suggestive. There has been a definite passive

* This work is being carried on under "The Crane Research Fund for the Study of Allergic Diseases in Children".

[1] Coca, A. F., *J. Allergy*, 1929, i, 74.

transfer of reagins from one human being to another. Coca attempted to transfer antibodies from a sensitive animal to the human skin but his series of experiments resulted negatively. However, Spain and Cooke[2] recently refuted these results by definitely transferring anaphylactic antibodies from the animal to the human skin.

In a paper by Ella Grove,[3] who worked in Coca's laboratory, she reports the attempt to transfer reagins to the lower monkey and chimpanzee. She succeeded in transferring this hypersensitiveness in 2 instances but in her discussion chooses to deny the validity of her results and concludes that the human reagin must be differentiated from the anaphylactic antibody.

The details of our experiments will be presented later.

In formulating our experiments we desired to parallel the situations in the human being and the lower animal. This experiment is difficult since many negative results are obtained because of factors which will be discussed in the complete report.

In these experiments we took the blood serum of a human asthmatic sensitive to horse dander and passively transferred this serum into normal guinea pigs and into normal human skin (B. R. and H. L. G.). 48 hours later these animals were injected with horse dander extract and several animals showed definite anaphylactic symptoms, in one instance anaphylactic death occurred. The transfer of the human serum to the normal human skin was positive in all instances.

In a second series of experiments, guinea pigs were sensitized with horse dander, not by parenteral injection, but by induction of respiratory anaphylaxis (asthma) in a manner previously described by us.[4] We took the blood serum of these animals 3 weeks later and transferred it to our skins intradermally and to normal guinea pigs intravenously. The transfer of guinea pig serum to the human skin resulted in a positive allergic skin reaction in certain instances. The passive transfer to the guinea pig gave positive results in certain instances.

In the human experiments, 6 asthma patients were used; 4 were negative, and 2 gave positive results. Of one of these patients, where blood was drawn at 2 different times, there was passive transfer to 6 of 15 animals, and in the second patient where blood was drawn at 3 different times, passive transfer was demonstrated in 5 out of 9 animals. Positive uterine contraction was obtained in

[2] Cooke, R. A., and Spain, W. C., *J. Immunol.*, 1929, xvii, 295.

[3] Grove, E., *J. Immunol.*, 1928, xv, 3.

[4] Ratner, B., Jackson, H. C., and Gruehl, H. L., *Am. J. Dis. Child.*, 1927, xxxiv, 23.

one. Positive transfer to the human skin was accomplished in these latter 2 cases.

In the animal experiments, in 14 animals in whom asthma was produced, there was one passive transfer to the human skin, and 3 suggestive transfers. In the passive transfer to normal guinea pigs there were 2 positive and 7 suggestive reactions in 16 animals injected.

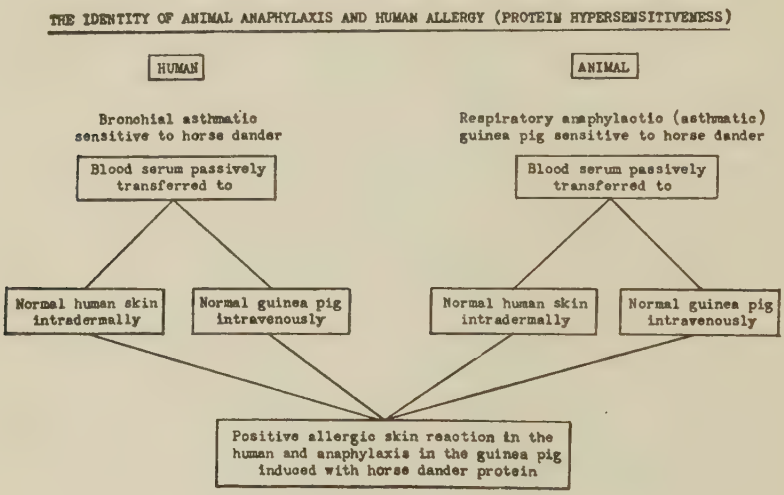

FIG. 1.

In Fig. 1 the results are correlated and show that the blood of a human asthmatic and the blood of an asthmatic guinea pig can passively sensitize the normal human skin and normal guinea pigs.

We deem our results indicate that hypersensitiveness in man and anaphylaxis in the lower animal are fundamentally dependent on a common anaphylactic antibody and that the distinction between anaphylactic antibody and the atopic reagin is untenable.

4863

Adsorption of Glucose Galactose Mixtures in the Intestine.

HARRY SOBOTKA AND MIRIAM REINER. (Introduced by Louis Gross.)

From the Laboratories of the Mount Sinai Hospital, New York City.

Plant and animal membranes exhibit selective permeability for chemically related substances. The diffusion of simple carbohydrates through the glomerular membrane of the kidney, through the

intestinal wall and through the membrane of monocellular organisms has been the subject of a great number of investigations. In several instances, paradoxical phenomena were observed, *e. g.*, that glucose and fructose are taken up by yeast at the same rate when offered singly, but glucose is preferred from a mixture of the two. Chemical reactions following the entry of the substances into the cell are responsible for this unusual behavior. The sugar absorbed from the intestinal wall is carried away subsequently through the mesenteric circulation and does not undergo chemical changes before reaching the liver. On the other hand, in the yeast cell, where the sugar reacts chemically in the immediate proximity of the membrane, the reaction products of the one substance may influence the diffusion of the other one.

In a series of experiments, Cori[1] and Nagano[2] established the absorption coefficients of various monosaccharides in the intestinal tract of the rat. These coefficients referring to glucose as 100, are 110 for galactose, 43 for fructose, 19 for mannose, etc. But from a mixture of equal parts of galactose and glucose, glucose was preferred at a rate of 100:68.[3]

We tried to repeat Cori's experiment using polarimetric analysis. Cori's criticism of acute experiments is justified as to the absolute amounts absorbed because of the influence of narcosis and laparatomy on the rate of absorption and because of the variations of body weight and intestinal surface used. However, as we were interested only in the relative rate of absorption of glucose and galactose, we found it most convenient to use the isolated intestinal loop of the rabbit.

Animals fasted 48 hours were narcotized with ether, a loop of the small intestine was clamped off and 50 cc. of 10% glucose-galactose mixture was injected into the intestinal lumen. Three samples were withdrawn after given intervals and analyzed polarimetrically and by Hanes'[4] modification of the Hagedorn Jensen method. For the last sample all the remaining fluid was withdrawn from the loop. The total volume was greater than the amount injected because the hypertonic sugar solution attracted water from the tissues.

The reduction values were interpolated from glucose and galactose curves obtained by Hanes' method. The $[\alpha]_D^{20}$ of a 10% solution of glucose and galactose, $52.5° + 81.1°/2 = +66.8°$, was verified. The $[\alpha]_D^{20}$ of the solutions removed varied between

[1] Cori, C. F., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 495.

[2] Nagano, J., *Pflüger's Arch. f. ges. Phys.*, 1902, xc, 389.

[3] Cori, C. F., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 290.

[4] Hanes, C. S., *Biochem. J.*, 1929, xxiii, 99.

TABLE I.

Time min.	Sugar Solution withdrawn cc.	% sugar	$[\alpha]_D^{20}$	% glucose total sugar	Glucose gm.	Galactose gm.	Total gm.	Remarks
0	50*	10.0	66.8°	50.0	2.50	2.50	5.00	Rabbit 1
25	18	6.24	66.0°	53.0	0.59	0.53	1.12	2.6 kg.
50	17	4.14	65.2°	55.5	0.39	0.31	0.70	loop=40
75	37	4.17	67.1°	49.0	0.75	0.79	1.54	cm.
Total unabsorbed					1.73	1.63	3.36	rate
Total absorbed				47.1	0.77	0.87	1.64	**100:113**
0	48*	10.0 †	67.85°	46.0	2.21	2.59	4.80	Rabbit 2
25	15	6.43†	68.7°	43.0	0.42	0.55	0.97	2.2 kg.
60	12	5.08†	66.35°	51.5	0.31	0.30	0.61	loop=50
85	30	3.82†	67.3°	48.0	0.55	0.59	1.14	cm.
Total unabsorbed†					1.28	1.44	2.72	rate
Total absorbed				44.7	0.93	1.15	2.08	**100:123‡**
0	50*	10.0	66.8°	50.0	2.50	2.50	5.00	Rabbit 3
30	18	2.48	67.0°	49.0	0.22	0.23	0.45	2.0 kg.
70	19	1.92	64.5°	53.0	0.21	0.15	0.36	loop=80
85	25	2.72	67.2°	48.0	0.33	0.35	0.68	cm.
Total unabsorbed					0.76	0.73	1.49	rate
Total absorbed				48.3	1.74	1.77	3.61	**100:102**

* Injected. † By Bertrand.
‡ Rate of gl:gal 100:105 calculated on 50:50 mixture.

TABLE II.

Time (min.)	Total	mgm. in 100 cc. non-fermentable reducing	Glucose	Galactose (=increase of non-fermentable over fasting)
0	ear 126	29	97	0
20	" 181	62	119	33
20	portal 243	76.5	166.5	47.5
60	ear 95	68	27*	39
60	portal 260	62	198	33

* The drop of glucose to 27 mg./100 cc. in the peripheral circulation following prolonged ingestion of a non-fermentable sugar is remarkable. A similar observation in humans will be reported by Fischer and Reiner elsewhere.

+64.5° and +68.7°, corresponding to 55.5 and 43% glucose. In the last column of Table I are given the relative rates of glucose: galactose absorption.

Blood was withdrawn from the ear-vein and from a mesenteric vessel of rabbit 3 and analysed by the Hagedorn Jensen method before and after the removal of fermentable sugar according to Somogyi's procedure.[5]

Conclusion: The relative rate glucose :galactose is unity within the

[5] Somogyi, M., *J. Biol. Chem.*, 1927, lxxv, 33.

limits of error with a slight preference for galactose in accordance with Cori's experiments on ingestion of individual sugars. The reversion of this relation for glucose-galactose mixtures was not observed under our experimental conditions.

4864

Endogenous Stimulation of Albino Rat Fetuses.

A. W. ANGULO Y GONZALEZ. (Introduced by Henry H. Donaldson.)

From the Wistar Institute of Anatomy and Biology.

Studies upon the progressive development of muscular activity of albino rat fetuses, from 15 days to almost 21 days after insemination, have been made.

During this study it has been found that fetuses of about 18 days after insemination do not respond to tactile stimulation of the hands. But, on ligation of the umbilical cord they give a very characteristic waving of the hand shortly after the time of ligature. This waving of the hand has a short duration and is followed by strong body movements typical of fetuses of that stage of development.

Fetuses of about 19 days after insemination begin to respond to tactile stimulation of the hands, and about 19½ days after insemination one can obtain discrete reflexes from this member. Thus stimulation of the dorsal side of the hand causes an extension of the hand with spreading of the fingers, while stimulation of the volar side of the hand causes flexion of the hand with closure of the fingers.

At this stage (19½ days) one cannot arouse the fetuses by stimulation of the hind legs. But on ligation of the umbilical cord the first observable movement is flexion of the hind legs which is then followed by waving of the hands and finally the characteristic body movements of this age.

These observations seem to indicate that the motor nerve reaches these parts sooner than the sensory nerve, also that it is possible to stimulate directly by means of metabolites (CO_2) the centers that control these movements.

4865

Insusceptibility of the Albino Rat to Experimental Amyloidosis.

B. L. ROBINSON AND H. S. THATCHER. (Introduced by Barnett Sure.)

From the Departments of Anatomy and Pathology, School of Medicine, University of Arkansas, Little Rock, Arkansas.

Amyloid has been produced experimentally in white mice by various workers, among whom are Kuczynski,[1] Letterer,[2] Smetana,[3] and Jaffé.[4] Nutrose (sodium caseinate) was injected daily. Smetana also used a cheese diet. Lucke and Markeley[5] have shown the difference of susceptibility to amyloid in rats and mice. They were unable to produce amyloid in rats by the injection of casein, but produced it in 75% of the mice.

The present study is upon the susceptibility of adrenalectomized rats to amyloid production. The rats were injected daily with 3 cc. of a 3% nutrose solution. The cheese diet used consisted of American cheese with a small amount of bread or whole wheat. When not fed on the cheese the rats were given a maintenance diet: 1/6 milk powder, 5/6 whole wheat, and salt in a quantity of 2% of the wheat.

A series of mice was also used. The mice were injected daily

TABLE 1.—Rat Series.

No.	Operation	Days experiment begun after operation	No. Nutrose injections	No. days on cheese	Amyloid
1*	Left adrenalectomy	13	214	150	—
2	" "	13	12	none	—
3*	Double adrenalectomy	13	207	144	—
4	" "	13	2	none	—
5*	Left adrenalectomy	29	47	108	—
6*	" "	29	76	138	—
7	Double adrenalectomy	29	none	6	—
8	" "	29	none	6	—
9	None		231	168	—
10	"		225	162	—
11*	"		57	119	—
12*	"		65	127	—

*Infected.

[1] Kuczniski, M. H., *Virchow's Arch.*, 1922, ccxxxix, 185.

[2] Letterer, E., *Beitrage zur path. anat. u. z. allg. Path.*, 1926, lccv, 486.

[3] Smetana, H., *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 383.

[4] Jaffé, R. H., *Arch. Path. and Lab. Med.*, 1926, i, 25.

[5] Lucke, B., and Markeley, L. A., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 642.

with 0.5 cc. of a 5% solution of nutrose. Other mice were fed on a diet of American cheese to which a small amount of bread was added. They were given water. The injected and control mice were fed on cracked corn, lettuce, carrots, and bread. They were not given water. Further facts on the 2 series are brought out in Tables I, II and III.

There are included in Table I, 4 unoperated rats that received injections and were fed on the cheese. In 4 rats (1, 5, 11, and 12) Congo red was injected into the heart and the rats killed one hour later. This was done to discover beginning amyloidosis. The liver, spleen, and kidney were examined in all rats, and other organs in several. The tissues were stained with methyl violet, Mayer's method, I-reaction, and hematoxylin and eosin. A gradual loss of weight occurred in all rats. In 6 rats there was marked infection for many days. In 2 livers there was focal necrosis illustrating the effect of the infection. In none of these animals did amyloidosis occur.

TABLE II.—White mice. Nutrose injections.

No.	Remarks	No. of injections	Amyloid
1	Killed	31	—
2	Died	41	+
3	"	62	—
4	"	64	—
5	"	65	—
6	"	67	+
7	"	74	+
8	"	74	+
9	"	74	—
10	"	75	—
11	"	80	+
12	"	98	+
13	Killed	101	—
14	"	101	—
15	"	101	+

TABLE III.—White mice. Cheese Diet.

No.	Remarks	Duration in days	Amyloid
1	Died	20	—
2	"	29	—
3	"	34	—
4	"	42	—
5	"	48	—
6	Killed	90	—
7	"	90	—
8	"	90	—
9	"	102	—
10	"	102	—
11	"	102	—

As controls 5 mice were kept for 90 days and 4 mice for 102 days. No amyloid was found in liver, spleen, or kidney.

The mice reported in Tables II and III were examined for amyloid in liver, spleen, and kidney. The organs were stained with hematoxylin and eosin, and with methyl violet. Contrary to other workers no amyloid was found in the mice on the cheese diet. In the mice injected with nutrose amyloid was found in 46.6% of the cases.

These experiments illustrate the resistance of the white rat to amyloid production. Although nutrose injections produced amyloid in 46.6% of mice; it was ineffective in adrenalectomized rats, both double and single, and even in those rats where the factor of infection was added.

4866

A Method for Staining Unfixed Brain Tissue With Silver.

A. E. TAFT AND S. DEW. LUDLUM. (Introduced by J. H. Bodine.)

From the Gladwyne Research Laboratory, Gladwyne, Pa.

In attempting to find an agent that will stain fresh brain tissue, various dyes were tried out. Those which are ordinarily employed in routine staining were first experimented with, as methylene blue, eosin, cresylviolet, janus green, neutral red, gentian violet, alum hematoxylin, etc., as well as black writing ink. None of these gave the clearly defined outlines which were desired, and all gave almost no staining of nerve cell processes. Metal impregnations were next experimented with, in which the reagents used with fixed tissue were employed. Both gold and silver were tried out on fresh tissue following the prescribed technical staining formulae for fixed material, but without success. The nearest to desired results among these was obtained by putting a very small fragment of brain cortex in a weak solution of silver nitrate, followed by washing in photograph developer solution. Even this method proved inadequate. In looking for something further to use a bottle of argyrol was happened upon, and this was tried only as a matter of curiosity. The result was so much better than that obtained in any other way, that it seems of sufficient interest to present to others wishing to pursue similar means of study.

Microphotographs are presented showing high power appear-

FIG. 1.

A group of brain cells, showing many processes, stained by the method described. Rat.

FIG. 2.

A single cell stained with argyrol, as described. From the brain of a day-old white rat.

FIG. 3.
Fixed and embedded specimen. Cajal stain. Human spinal cord.

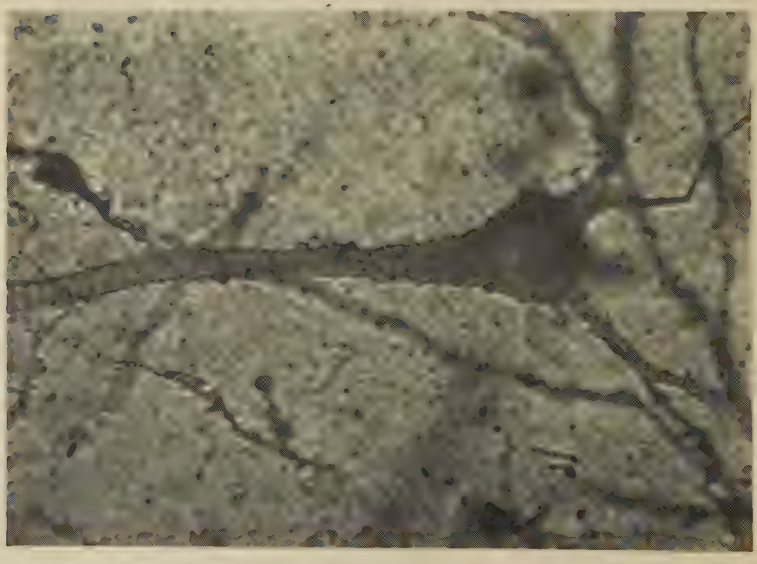

FIG. 4.
Fixed and embedded specimen. Golgi stain. Human brain cortex.

ances of the tissues examined, and to contrast with them similar pictures are included of brain tissue stained both by the Cajal and Golgi methods, after fixation and embedding. Our method has the advantage of no distortion of the protoplasmic structure. With the ultramicroscope, it has the same appearance as in fresh tissue.

The details of the method followed in using argyrol as a means of staining brain tissue are quite simple, and are as follows:

The top of the skull was removed from the head of a freshly decapitated chicken, or of an etherized rat, and the fresh brain tissue placed at once in 10% argyrol.

The length of time staining is continued seems not to be of importance after the first 6 to 24 hours; that is, tissue left in argyrol longer than 24 hours shows no better staining than at the end of that time.

The next step was to wash in distilled water. To do this a fragment is put in water and left indefinitely. No change is distinguishable after having been in water a period of several days.

From water, a very small fragment, not larger than half the size of an ordinary pinhead, is placed in a small drop of water on an ordinary glass slide, and covered with a number one cover glass. Slight pressure on the cover glass flattens the tissue fragment into a thin film which can then be examined with oil immersion as well as with low power. The appearances are shown in the accompanying microphotographs. These photographs were made with direct illumination, as the dark field has given unsatisfactory results with stained material. The black and white of unstained specimens, on the other hand, photograph equally well, as they appear in the ultramicroscope.

This brief consideration of the use of argyrol as a means of staining fresh nerve tissue is a preliminary report which will be enlarged upon by further work. Its aim is to suggest a means for the immediate study of fresh tissue at surgical or postmortem operations.

Acknowledgement is made to Professor Addison of the Department of Histology in the University of Pennsylvania for generously loaning the mounted sections stained by the Cajal and Golgi methods, which appear in the photographs.

4867

Relation of Weight of Placenta, Cord and Membranes to Weight of Infant in Normal Full-term and in Premature Deliveries.

S. B. D. ABERLE, W. R. THOMPSON AND E. H. PITNEY.
(Introduced by A. H. Morse.)

From the Departments of Obstetrics and Gynecology and of Pathology, Yale School of Medicine.

The correlation between the weight of the infant and the weight of the placenta, cord and membranes has been studied in 4129 instances. The instances were distributed in 2 main classes, one class consisting of fetuses weighing under 1500 gm. and the other class of infants weighing 1500 gm. or over. In the latter case males and females were analyzed separately.

In each case a positive correlation was found (approximately $r = 0.5$). A good fit to linear regression was observed, particularly in the case of infants weighing 1500 gm. or more. A critical survey was made of previously reported work in this field.

4868

The Form of the Electrocardiogram. I. Intrinsicoid Electrocardiographic Deflections in Animals and Man.

A. G. MACLEOD, FRANK N. WILSON AND PAUL S. BARKER.

From the Department of Internal Medicine, University of Michigan Medical School.

Wilson, Wishart, and Herrmann[1] have called attention to the laws which govern the flow of currents in solid conductors and to the fact that these laws determine the distribution of the potential differences produced by the heart beat within the body and at its surface, and have pointed out that leads in which one electrode is placed close to the heart and the other at a distance from it are semi-direct leads.

In experiments now in progress, it has been found that semi-intrinsic or intrinsicoid deflections may be obtained from the ventricles of the dog when the heart is covered by a pad of gauze, 1½ to 2 cm. thick, soaked in normal saline solution. Compared with

[1] Wilson, Wishart and Herrmann, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 276.

true intrinsic deflections, these deflections are smaller in amplitude and less steep when the recording instrument is used at the same sensitivity. Intrinsicoid deflections can also be obtained from the ventricular cavities by a blood contact. In the case of the auricles, intrinsicoid deflections cannot be obtained either through gauze of the thickness mentioned or from the auricular cavities. It is found that true intrinsic deflections from these chambers are rapidly degraded by increasing the size of the exploring electrode.

The difference between auricles and ventricles is due, so it is suggested, to the difference in the manner in which the excitation process spreads over the ventricular as compared with the auricular muscle.

When right bundle branch block is produced in dogs the intrinsicoid deflections obtained over the right ventricle are late; those obtained over the left ventricle are early. In left branch block the reverse is the case.

In man intrinsicoid deflections can be obtained by placing the exploring electrode upon the precordium. The few observations carried out thus far on patients with bundle branch block support the view expressed by Barker, Macleod, Alexander and Wilson[2] that the electrocardiograms at present attributed to right branch block are the result of left branch block.

Some observations made incidentally in the course of our experiments strongly suggest that so-called monophasic responses obtained in animals by placing one electrode upon an injured region of the cardiac surface and the other upon an uninjured region are the result, in so far as their monophasic character is concerned, of activity of the muscle immediately adjacent to the injured area, and not to the activity of the muscle beneath the electrode placed upon uninjured tissue. It is suggested that such curves are due to depolarization and repolarization of the injured tissues.

[2] Barker, Macleod, Alexander and Wilson, *Trans. Am. Assn. Am. Phys.*, 1929, xliv, 125.

4869

The Form of the Electrocardiogram. II. The Character of the Excitation Wave in Auricular Muscle.

FRANK N. WILSON, A. G. MACLEOD AND P. S. BARKER.

From the Department of Internal Medicine, University of Michigan Medical School.

Wilson, Wishart, and Herrmann[1] have pointed out that the distribution of the potential differences produced by the heart beat within the body and at its surface is determined by the laws which govern the flow of currents in a solid conductor within which a potential difference is maintained.

In the case of a conductor of infinite extent the potential of any point (V) is determined by the following expression:

$$V = c\left(\frac{1}{r_1} - \frac{1}{r_2}\right). \quad (1)$$

In this expression (c) is a constant, (r_1) is the distance of the point from the positive pole or source, and (r_2) is the distance from the negative pole or sink.

Let us assume that the positive pole is located at a point of which the coordinates are (a), (0), (0), and the negative pole at a second point (—a), (0), (0), and let us investigate the line, $y = b$, $z = 0$. The expression

$$V = c\left(\frac{1}{\sqrt{(x+a)^2 + b^2}} - \frac{1}{\sqrt{(x-a)^2 + b^2}}\right) \quad (2)$$

will then give the potential of any point on this line.

Let us imagine that we can examine the potential of this line with the string galvanometer. To do so let us connect the left-hand electrode to a point so far distant from the origin that it may be considered as indifferent, and let us move the right-hand electrode with a uniform velocity along the line mentioned from a point where (x) has a very large positive value to a point where (x) has a very large negative value.

The form of the curve which would be written by our galvanometer under these conditions can be determined by equation (2), of which the essential constants are assumed to be known.

Let us now imagine that the exploring electrode is stationary and that the system of coordinates approaches it with the same uniform

[1] Wilson, Wishart and Herrmann, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 276.

velocity as before. The recorded curve will then be identical with the previous one.

By placing the right-hand electrode upon the right auricle of the dog at a point distant from the sinus node and the left-hand electrode at a point distant from the heart, electrograms identical in all essential particulars with the curves plotted from equation (2) are obtained. Knowing the velocity of the excitation process in the auricle the effective distance (2a) over which the excitation wave extends may be determined. It is very short, apparently 10 mm. or less. We conclude from such experiments that auricular muscle produces electrical effects during the period of activation and deactivation only. When fully active it exerts no influence upon the electrocardiogram. The period of activation at any point probably lasts 0.01 sec. or less.

The process of deactivation produces a curve similar to that produced by activation except that the phases of the curve are reversed, the amplitude is less and the effective distance (2a in formula 2) over which the wave of deactivation extends is greater than in the case of activation.

4870

The Form of the Electrocardiogram. III. Opposed Potential Differences.

FRANK N. WILSON, A. G. MACLEOD AND PAUL S. BARKER.

From the Department of Internal Medicine, University of Michigan.

At the onset of ventricular systole the excitation process is spreading within the ventricular muscle in many different directions simultaneously. Many of the electrical forces, or potential differences, produced are opposed by forces opposite in direction, and their effects are consequently neutralized. Other potential differences are not opposed; it is these potential differences and these alone which have an effect upon the electrocardiogram.

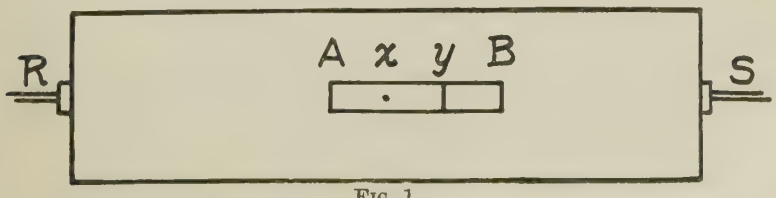

FIG. 1.

Consider the simple strip of muscle AB (Fig. 1) immersed in a large body of physiological saline solution or other similar conducting material. Place one electrode at *R,* and a second electrode at *S* and connect these electrodes to a string galvanometer in such a way that relative negativity at *R* will cause an upward deflection when the electrogram of the muscle is recorded. Given that *RX* equals *SX* (Fig. 1). Stimulate this muscle at *X;* given that XA equals XY. It is obvious that the excitation process will spread toward *A* and toward *Y* simultaneously; that the potential differences produced by the spread in one direction will be completely neutralized by the spread in the opposite direction and that no deflection will result until that part of the muscle lying between *A* and *Y* has completed its period of activation. Such deflections as do occur will result from activation of that part of the muscle lying between *Y* and *B.*

If instead of the strip of muscle *AB* we have a circular sheet of muscle and stimulate this muscle at its center, the center being equidistant from *R* and *S,* the excitation process will travel radially in all directions and since no unopposed potential differences will be produced no deflections whatsoever will occur. The same result will be obtained from a spherical shell of muscle if all points on the interior surface of the shell are stimulated at the same instant.

Each of the cardiac ventricles may be considered a spherical shell of muscle from which a segment at the ventricular base has been removed. If the ventricular walls were of uniform thickness and the entire endocardial surface was activated at all points simultaneously, all of the potential differences produced by each ventricle would be neutralized except those resulting from activation of the region at the apex opposite the opening in the spherical shell at the base.

The observations made by Lewis and Rothschild[1] indicate that all points on the endocardial surface of both ventricles, except points on both sides of the upper septum and possibly points on the endocardial surface of the conus, are activated before the inscription of *R* in lead II begins.

When one electrode is placed upon the ventricular surface and the other at an indifferent point an intrinsic deflection is obtained. The onset of this deflection indicates that the excitation wave, traveling from within outward, has reached the epicardial surface. Since, as pointed out in article II of this series[2] the excitation process extends over a very short distance, the appearance of an intrinsic deflection

[1] Lewis and Rothschild, *Phil. Trans. Roy. Soc.,* 1915, ccvi-B, 328.

[2] Wilson, MacLeod, Barker, PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 588.

under the above conditions indicates that the subjacent muscle has been completely activated and is, therefore, no longer producing potential differences. If potential differences directly opposed to those which were produced by the activation of this subjacent muscle, now complete, are still existent they will produce an effective potential difference of such a character as to indicate relative negativity on that side of the body on which the investigated point lies.

Considerations such as these lead us to conclude that *R* of the normal human electrocardiogram is produced by unopposed potential differences resulting from activation of the apical regions of the heart, and that the *S* deflection is produced by unopposed potential differences resulting from activation of the thick ventricular walls at the cardiac base.

They also lead to the conclusion that in bundle branch block the average spread of the excitation wave during the inscription of the chief initial deflections is from the contralateral toward the homolateral side.

We have also applied these considerations to the analysis of the curves which depict ventricular preponderance and to the analysis of the *T*-deflection of the electrocardiogram.

4871

The Form of the Electrocardiogram. IV. The Mean Electrical Axis and the Center of Stimulation.

FRANK N. WILSON, A. G. MACLEOD AND P. S. BARKER.

From the Department of Internal Medicine, University of Michigan Medical School.

Just as a material body may be considered the sum of an infinite number of material particles differing from each other only with respect to their orientation in space, so the ventricular muscle may be considered the sum of an infinite number of units differing only with respect to the direction in which the excitation process passes over them, and consequently in the direction of the potential differences which they produce during ventricular activation.

The analysis of the electrocardiogram is greatly complicated by the fact that not all of the muscle units are activated at the same time. This difficulty may be eliminated by determining the mean deflection in each of the 3 standard leads over any desired interval. The mean deflection in lead *I* (Me_I) during the QRS interval may be expressed by the following equation:

$$Me_I = \frac{1}{\text{QRS-Int.}} \int_S^Q (QRS)\, dt.$$

In this equation the value of the integral is the area under the curve, determined by planimeter, between the onset of *Q* and the end of *S*.

If the mean deflection for any interval be determined for each of the 3 standard leads in this manner, the mean electrical axis and the mean manifest potential difference may be obtained by means of Einthoven's triangle. In this way the electrocardiogram, so far as the interval in question is concerned, may be reduced to a single vector.

The mean electrical axis during the inscription of P will give the average direction of the excitation process in the auricles. The mean electrical axis during the QRS interval will give the average direction of the excitation process in the ventricular muscle. The mean electrical axis during the inscription of T will give the average direction of the wave of ventricular deactivation. The electrocardiogram will thus be reduced to 3 simple vectors.

In the case of a homogenous mass of excitable tissue immersed in a large body of non-excitable medium having the same electrical conductivity, it may be shown that the total electrical effect, so far as points distant from the mass are concerned, may be represented by a single vector drawn from the point of stimulation to the center of mass, provided only that the mass is of such a form that the excitation process will reach every point within it by radial spread. The vector mentioned will give the direction of the average spread of the excitation process and its length will be a function of the magnitude of the total electrical effect.

Each of the cardiac ventricles is lined by Purkinje tissue, which conducts the impulse with great rapidity as compared with the ordinary ventricular muscle. The spread of the excitation wave through the ventricular walls is, therefore, much the same as if the excitation wave spread radially from a point within the ventricular cavity. This imaginary point may be called the center of stimulation. The mean electrical axis during the inscription of QRS will then point from the center of stimulation toward the center of mass of the ventricular muscle. The total electrical effect, or the value of the integral in equation (I), will be a function of the distance between these 2 points.

4872

Changes Produced by Sugar Solutions in Hypotonic Hemolytic Systems Containing Red Cells of Man.

J. FRANKLIN YEAGER. (Introduced by Eric Ponder.)

From the Washington Square College, New York University.

The inhibitory effect of sucrose solutions upon saponin and taurocholate hemolysis has been studied in a previous paper.[1] The present report deals with the changes produced by solutions of sucrose and other sugars in the resistance of the hypotonic hemolytic system:

(a) — c.cs. M/3.6 sugar.
(b) — c.cs. 0.80% NaCl.
(c) — c. cs. distilled water.
(d) 0.40 ccs. ½ standard red cell suspension.

The quantities of (a), (b) and (c) are made such that the resulting system is of the hypotonicity desired. "Resistance" is expressed as that tonicity of the system which will just produce complete hemolysis in one hour.[2]

A typical experiment is presented. In Fig. 1, Curve A shows the change in resistance of the system when increasing quantities of 0.80% NaCl are replaced by equal quantities of M/3.6 sucrose, the cell suspension being a citrate-NaCl-NaCl suspension; similarly, Curve B shows the effect of increasing quantities of M/3.6 sucrose, when the suspension is a citrate-sucrose-sucrose suspension. These curves indicate that the replacement, in this way, of NaCl solution by sugar solution in the system produces at least two effects: (1) an immediate effect, resulting in an increased resistance of the system and (2) a more prolonged effect, resulting in a comparative decrease of resistance. Curve A represents the first effect only. In Curve B, the first effect is superimposed upon the second; here the immediately produced increase in resistance occurs in the case of red cells in which a comparative decrease of resistance has already been brought about by a more prolonged contact with the solution of sugar. Essentially the same results are obtained with M/3.6 maltose and M/3.6 lactose, which increase resistance, and M/3.6 dextrose, which decreases the resistance of the system. These experiments, together with observations of the following type, indicate that the destruction of red cells by a hypotonic hemolytic system involves to a con-

[1] Yeager, J. F., *Quart. J. Exp. Physiol.*, 1929, xix, 219.
[2] Ponder, Eric, *Biochem. J.*, 1927, xxi, 56.

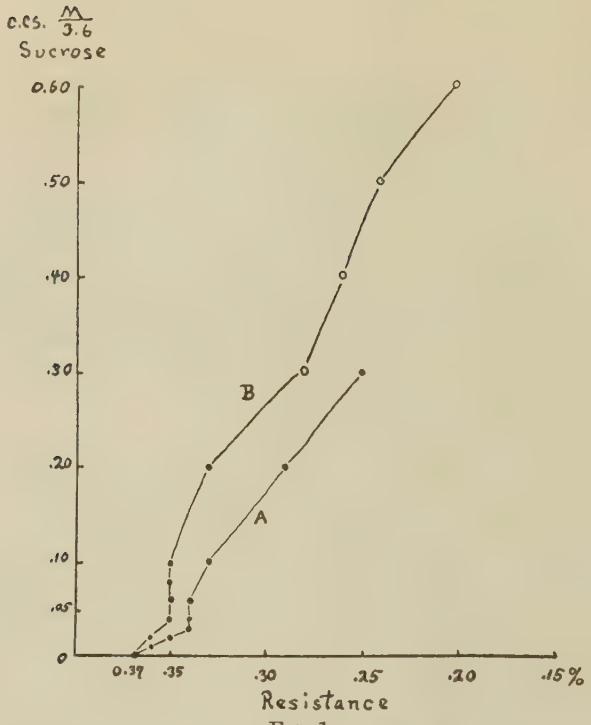

FIG. 1.

Curve A: NaCl suspension. Curve B: dots—suspension of NaCl and sucrose in varying proportions; circles—suspension of 1 part of 0.80% NaCl to 3 parts of M/3.6 sucrose.

siderable extent factors other than those of pure osmosis: (a) red cells suspended for 3 hours in M/3.6 lactose show a resistance of less than 0.20%; (b) red cells suspended in 0.80% NaCl and kept for 36 hours at 10° C. show a resistance of less than 0.20%; (c) in both cases, microscopic observation of a hypotonic NaCl system that just fails to completely hemolyse shows that the few remaining cells are not swollen into spheres, as might be expected from an hypothesis involving the occurrence of only osmotic changes, but are mostly crenated, cup-shaped and disc-shaped; (d) the resistance (as defined above) is altered by altering the rate at which the suspension is added to the system.

4873

Lack of Antigenic Power of a Highly Purified Diphtheria Toxin and Detoxification by Ultraviolet Light.

HENRY WELCH AND EMERSON MEGRAIL.

From the Department of Hygiene and Bacteriology, Medical School, Western Reserve University.

The literature concerning the effect of ultraviolet radiation of toxins has been summarized by Norton[1] with the statement that "In the medium in which bacterial toxins are found or are produced they appear to be relatively stable toward ultraviolet light." The work of Lowenstein,[2] and of Hortock, Schurman and Stiner,[3] which led to this conclusion, was done with the usual broth filtrates of organisms and in the case of diphtheria toxin from 6 hours to 20 days radiation was necessary to produce marked lessening of the toxicity of the filtrates. The former investigator was unable to produce an active immunity with radiated toxin. Although a previous investigation[4] had demonstrated the marked absorption of ultraviolet rays between 2000 and 3100 A° by proteins in solution or in suspension, it was thought advisable to determine whether the longer rays from "C" carbons (National Carbon Co.) would penetrate sufficiently to detoxify a broth filtrate of the diphtheria bacillus. Using the apparatus described in a previous article,[5] various dilutions of this toxic broth were radiated at varying distances and for varying periods of time, it was found that detoxification could be brought about but the long period of exposure necessary made it of little practical significance.

A water-clear, colorless diphtheria toxin prepared by the method of Gross[6] and with his aid, was found to be extremely toxic. Toxin prepared in this way contains but little protein. Since it had been found that the period of radiation necessary to insure detoxification varied directly with the potency of the toxin, a dilution of this toxin in physiological salt solution was selected for radiation, which gave a strong intracutaneous reaction in the guinea pigs. This purified

[1] Norton, J. F., "The Newer Knowledge of Bacteriology and Immunology." Univ. of Chicago Press, 1928, 371.

[2] Lowenstein, E., *Z. Exp. Path. u. Therap.*, 1914, xv, 279.

[3] Hortock, O., Schurman, W., and Stiner, O., *Z. Imm. u. Exp. Therap.*, 1914, xxi, 643.

[4] Welch, H., and Perkins, R. G., *J. Prev. Med.*, 1930, iv, 15.

[5] Perkins, R. G., and Welch, H., *J. Prev. Med.*, 1929, iii, 363.

[6] Gross, P., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 696.

toxin was found to be destroyed by the action of "C" carbons in 2 minutes, at a distance of 25 cm. When 3% glycerine, having the same pH (7.8) as the purified toxin, was added it was found that detoxification was brought about in 4 minutes.

To test the antigenic power of Gross' toxin and of the detoxified glycerinated and unglycerinated preparations of this toxin, 6 guinea pigs were given 10 injections at intervals of 5 days with each of these 3 products. Twelve days after the last injection half of each group of animals was given intracutaneous injections of 1/40 and 1/50 M.L.D. of ordinary diphtheria toxin. All showed positive reactions, and when these animals were given 1 M.L.D. of toxin subcutaneously, 2 days later, all died. The remaining animals were tested likewise 30 days after the last injection and were also found to be non-immune.

Diphtheria toxin prepared by the method of Gross is easily destroyed by ultraviolet light. Since, however, this purified toxin itself is *not antigenic,* a radiated non-toxic preparation could not be expected to be antigenic. It is apparent that in the preparation of this toxin the antigenic fraction is lost while the toxin remains. Evaluation of this point will necessitate further work, which, we understand, is to be carried out by the same group which first prepared the toxin.

Since investigation of ricin (to be published at an early date) has indicated that it is possible to detoxify this product with ultraviolet light and still retain its antigenicity, the results noted above do not obviate the same possibility with diphtheria toxin and other toxins under the proper conditions.

4874

Histology of the Anterior Pituitary of the Foetal Pig with Reference to Growth and Maturity.

W. O. NELSON. (Introduced by Eric Ponder.)

From Washington Square College, New York University.

It is well known that the pars anterior of the hypophysis cerebri is made up of a variety of cell types, *viz.*, eosinophils, basophils, and chromophobes (Flesch,[1] Kraus[2]). The presence of at least 2 func-

[1] Flesch, M., *Tagebl. d. 57 Vers deutsch Naturf. u. Arzte. Magdeburg,* 1884, 195.

[2] Kraus, E. J., *Zeigler's Beitrage,* 1914, lviii, 159.

tions attributable to the pars anterior is of interest in view of these cell types, and it is logical to suppose that a relation may exist between the different types and functions.

Dortzbach and Smith[3] recently have studied the effect of anterior lobes, obtained from foetal pigs, when introduced intra-muscularly into the immature mouse. They employed a graded series and found that the 10 cm. stage was the earliest at which the growth hormone was present in sufficient quantity to evoke a positive response to their test. Anterior lobe material from 20 cm. pigs produced a positive response to the maturity reaction. These results indicate the existence of a time difference in the attainment of the threshold values necessary to cause a response to the biological tests employed by these authors.

It seemed advisable to make a histological study of a graded series of foetal pig pituitaries in order to determine whether any relation exists between the physiological phenomena described by Dortzbach and Smith and the order of appearance of the cell types characteristic of the pars anterior. A graded series was obtained and the 10 and 20 cm. sizes were selected as critical stages. The entire pituitary in each instance was carefully dissected out and fixed in Zenker's fluid. All material was sectioned 5 micra in thickness and stained with eosin and Delafield's haematoxylin.

The histological picture characteristic of the 10 cm. stage was decidedly a basophilic one. A few scattered eosinophils were present but their scarcity seemed to indicate that the dominant element, at least in a morphological sense, is the basophilic cell.

The 20 cm. stage disclosed a distinctly different picture. Many eosinophilic elements were in evidence, some clumped together to form the so-called "nests". Basophilic cells were present in considerable number, though less numerous than the eosinophils.

The evidence so far obtained points toward the existence of a distinct histological difference, which may be related with the physiological one found by Dortzbach and Smith, in the pars anterior of the foetal pig at 10 and at 20 cm. The preponderance of basophilic elements in the earlier stage is altered to an eosinophilous dominance in the older foetuses. Further work in obtaining a series from an early age up to birth is in progress.

[3] Dortzbach, C., and Smith, P. E., *Anat. Rec.*, 1929, xliii.

4875

Effect of Ultraviolet Light Upon Genus Trichophyton.*

ERNEST L. SCOTT AND EARL B. MCKINLEY.

From the School of Tropical Medicine of the University of Porto Rico under the auspices of Columbia University and the Department of Physiology, College of Physicians and Surgeons, New York City.

Ring-worm infection is very common in tropical countries but is also quite prevalent in temperate climates. The genus trichophyton seems to be widespread over the face of the earth. Sabouraud lists some 30 species. These are differentiated chiefly by cultural characteristics, morphology, pigment formation, character of the lesions produced and the host affected. Ring-worm infection is very persistent and frequently responds only to the most vigorous treatment. One of us (McK.) has observed cases in the Philippines which have persisted for fifteen to twenty years, the lesions appearing and disappearing periodically during this time. Patients have stated that sojourns in colder climates are frequently accompanied by disappearance of the lesions which promptly return when they again take up residence in the tropics.

It has been thought that these fungi are very resistant to ultraviolet light since they produce lesions on parts of the body normally exposed to sunlight (particularly in animals) as well as other parts (in man) which are protected by clothing. However, both the X-ray and the ultraviolet rays have been used empirically for the treatment of some of these conditions in man.

We have recently tested the effect of ultraviolet light upon the *Trichophyton asteroides,* a variety which affects chiefly horses and cattle and gives rise to inflammatory lesions with folliculitis and formation of kerion, the clinical picture of which has usually been designated *Herpes tonsurans.* The fungus was grown on Sabouraud and after a growth measuring 8 cm. was obtained it was lifted off and ground up in a sterile mortar with sterile sand and suspended in physiological saline. To quartz tubes containing 2 cc. of saline a small quantity (0.1 cc.) of the fungus suspension was added. Six tubes were prepared in this manner including a control. Two ordinary test tubes containing glucose broth were also similarly inoculated. The quartz tubes 1, 2, 3, 4 and 5 were then exposed to ultraviolet light as generated by the Alpine sun lamp at one foot dis-

* This work is part of a general study on fungus infections which is being supported by the Ella Sachs Plotz Foundation.

tance for 5, 10, 15, 20 and 30 minutes respectively. At the end of the experiment 5 cc. of glucose broth were added to each tube. All the tubes, including the 3 controls, were left at room temperature for several days. Growth of the fungus did not occur in any of the tubes containing the fungus which were exposed to ultraviolet light but luxuriant growth was obtained in all of the controls.

Under the conditions of these experiments it would seem that at least one member of the Gypseum group of ring-worm fungi *(Trichiphyton asteroides)* is markedly susceptible to the action of ultraviolet light. Probably other members of the genus are also susceptible, though such tests as we have employed are more qualitative than quantitative. The rationale of treating this type of infection with ultraviolet light is apparently borne out by the experimental method.

4876

Experimental Aortic Insufficiency. I. Regurgitation Maximum and Mechanisms for Its Accommodation Within Mammalian Ventricle.

CARL J. WIGGERS AND HAROLD GREEN.

From the Department of Physiology, Western Reserve University Medical School, Cleveland, O.

The investigations reported in this paper were designed to determine the greatest magnitude of regurgitation possible in the relaxing mammalian heart under optimal conditions and to study the mechanisms by which such volumes are accommodated. These questions have previously been studied chiefly with the aid of artificial circulation machines. But, as Allan[1] properly concludes, conditions are manifestly so different in the mammalian heart that it would be unwise to transfer such values to the human circulation. A number of physical factors enter into relaxation of the mammalian heart which are difficult to reproduce in an artificial model. Ventricular diastole begins with a phase of *isometric relaxation* during which the intraventricular pressure is reduced from that existing in the aorta to a level below that in the auricle. During this early diastolic phase, averaging 0.07 second in the dog and 0.12 second in man, the ventricular cavity remains in the state of obliteration reached at the end of ejection. Consequently, by far the greatest

[1] Allan, *Heart*, 1926, xii, 200.

backflow and possibly all of it must occur during the subsequent inflow phases. Whether the rapid filling from the auricles hinders aortic regurgitation or, *vice versa,* whether regurgitating blood re-

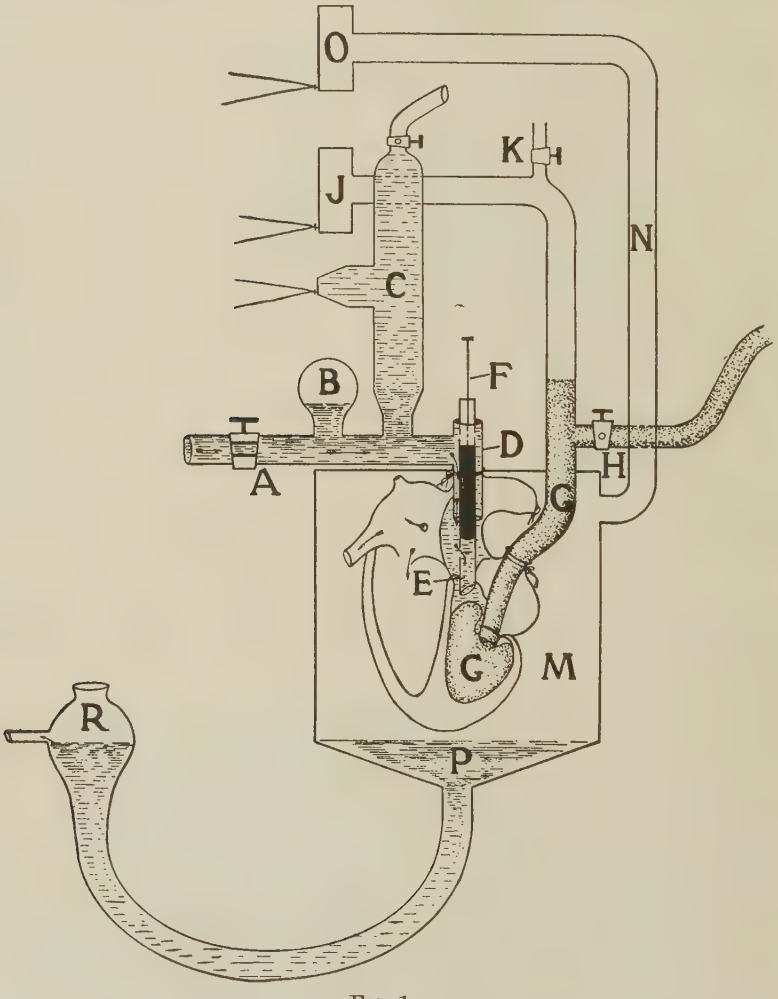

FIG. 1.

Scheme of apparatus. A, arterial system connected to flasks of Tyrode solution under pressure; B, elastic compression chamber; C, optical manometer; D, cannula for perfusing coronaries via ligated aorta; E, trochar cannula allowing regurgitation through side opening when plunger (F) is withdrawn as shown.

G-G, tidal volume inclosed in rubber bag within ventricle and in tube; H, side-cock by which fluid level (G) may be altered; J, segment capsule recording volume of tidal blood; K, side-cock for equalizing pressures.

M, chamber acting as cardiometer for heart and connected by tube (N) to a segment capsule (O) for recording volume changes; P, fluid dripping from right heart after passing through coronary system kept at constant level by compensator (R).

duces the filling from the auricles remains a debatable question. The extent to which a backflow from the aorta is accommodated by replacing inflow from the auricle or by stretching of the ventricle has also not been satisfactorily settled.

Procedure. Our experiments were carried out on cats' hearts arranged to contract isometrically and supplied through the aorta and coronary vessels with nutrient fluid under adjustable pressure. The special arrangements consisted (1) in the introduction of a plunger cannula through the aortic valves for the purpose of producing an aortic insufficiency at will, and (2) in the insertion of a thin rubber balloon into the left ventricle by way of the mitral orifice. In this way, the tidal volume was confined to a closed system and its mixture with the regurgitating fluid was prevented. The changes in aortic pressure, the variations in the tidal volume and, finally, the alterations in the capacity of the heart itself was simultaneously recorded by suitable optical methods. The details of the apparatus should be sufficiently clear from the sketch of Fig. 1. Two technical considerations must, however, be mentioned briefly: Changes in the vigor of contraction due to prolonged lowering of arterial pressure after production of an insufficiency were prevented by limiting our

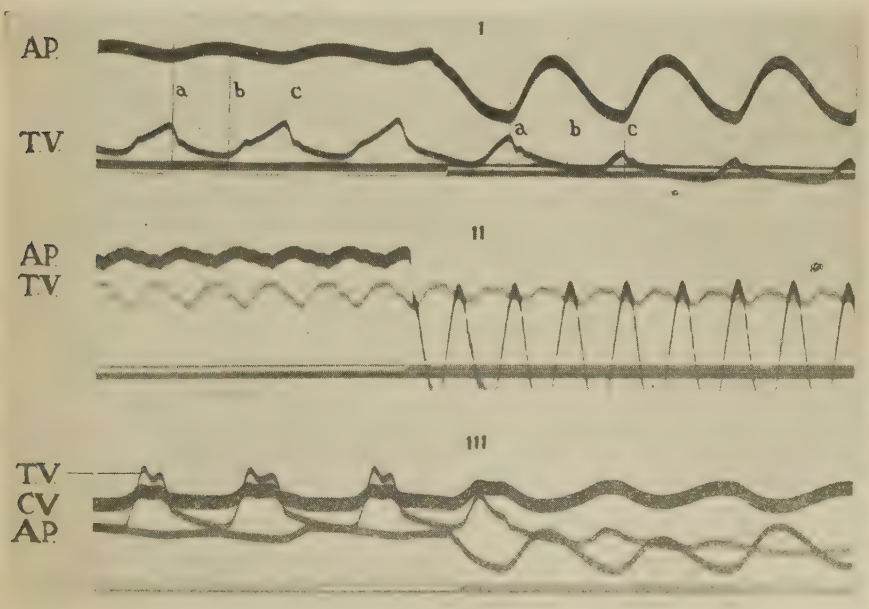

FIG. 2.

Records from 3 experiments showing effects of aortic insufficiency. I, from Exp. 13, X; II, from Exp. 12, VI; III, from Exp. 15, VII.

A.P., arterial pressure; T.V., tidal volume; C.V., cardiometric volume.

observations to 5 or 10 beats after the insidence of a lesion. In order to assure the most favorable conditions for regurgitation—*i. e.*, to recreate the conditions obtaining in the severest clinical instances in which the size of the aortic ring approaches that of the a-v opening—we kept the tubes passing through the aortic and mitral openings identical in size (surface area = 7.1 mm.^{2}).

It should be emphasized that the percentage regurgitation in naturally beating hearts may be considerably less than in our preparation, but that it probably could not be greater.

Results: Many tests were made on 20 different cat hearts. Records from 3 such tests are reproduced in Fig. 2. In each, a portion of the normal records precedes the sudden production of an aortic insufficiency. In the upper set of tracings (I), are shown the changes in aortic pressure (AP), and in tidal volume (TV) entering and leaving the balloon. Previous to the lesion, the aortic pressure variations though recorded with an optical manometer were not very great owing to the fact that the trochar almost entirely filled the aortic ring. The curve of tidal volume changes (TV) presents the gross features of a ventricular volume curve. During systole (ab), the curve at first moves downward rapidly, then, more slowly; during diastolic filling (bc), it first rises with a rapid, then with a slower gradient. When a lesion is produced, the natural diastolic inflow is encroached upon; less fluid enters the balloon and as a result the tidal volume expelled decreases to 60% of its normal volume. These records were taken under conditions highly favorable for regurgitation; the arterial pressure previous to regurgitation was high (mean 95 mm. Hg.) and the venous inflow pressure was very low (10.5 cm. saline). Would the tidal volume be equally reduced if the arterial pressure were lower, or the venous pressure higher? We tested this in many experiments but found no essential difference.

The second set of records (II) in Fig. 2 illustrate the effects obtained when arterial pressures were rather low (mean = 40 mm. Hg.) and the inflow pressure was very high (210 cm. saline). The tidal volume curve (TV) again shows a considerable decrease, owing to an impaired diastolic filling. In this instance diastolic volume and tidal volume were decreased 50%.

Before we may conclude that the difference of 40 or 50% signifies a regurgitation of this magnitude it is necessary to demonstrate that neither the vigor of contraction nor the diastolic size of the whole ventricle altered. The actual regurgitation may actually be larger due to an additional stretching of the ventricles; or it may be

smaller owing to diminished vigor of ventricular contraction. Information on both of these possibilities was obtained in the later experiments by recording, in addition, the volume changes of the entire ventricle (cf. Fig. 1). Such triple records are shown in the third tracing (III) of Fig. 2. The changes in aortic pressure (AP) and in tidal volume (TV) are similar to those shown in the other tracings. The cardiometric curve (CV) of the ventricles indicates that the amplitude of excursion slightly increases; hence, the coincident diminution in the tidal volume cannot be due to less vigorous contractions. On the other hand, we note a rise of the base line equal to about 10% of the normal variation. An additional 10% of regurgitated blood is obviously accommodated by stretching the ventricles. This is approximately the value also found in other tests.

Summary: The maximum aortic regurgitation possible under optimum conditions in the mammalian heart was studied by means of a perfused cat's heart arranged to contract isotonically and in such a manner that the tidal volume entering and leaving the ventricle was kept separate from the regurgitating fluid. The difference between tidal volume before and after a lesion offers an estimate of the volume which regurgitates due to interference with natural filling from the auricles. The increase in the external size of the ventricles indicates the percentage regurgitation made possible by stretching the ventricular myocardium.

Our results show that under optimum conditions the total regurgitation can equal 50-60% of the normal tidal volume in the perfused heart. Of this 40-50% is accommodated by replacing a natural inflow from the auricle and only 10% by additional stretching of the ventricular myocardium.

We believe that the percentage regurgitation could not possibly exceed these values in naturally beating intact hearts; though it may be considerably less.

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Isolation of the Relaxative Hormone on the Corpus Luteum.*

H. L. FEVOLD, FREDERICK L. HISAW AND R. K. MEYER.

From the Department of Zoology, University of Wisconsin.

Pelvic ligaments of guinea pigs undergo very pronounced relaxation during pregnancy to facilitate the birth of young. While studying the physiology of this reaction, one of us discovered that it was under hormonal control and that the substance responsible was present in the blood of certain mammals during pregnancy.[1] This hormone is capable of producing relaxation of the pelvic ligaments of virgin guinea pigs in a fashion typical of pregnancy, if given while the animals are in or recovering from oestrum. Later it was found that this reaction could also be produced through the use of extracts of corpora lutea of sows' ovaries. General methods for the preparation of these extracts have been given elsewhere.[2] These corpus luteum extracts, in addition to relaxing the pelvic ligaments, also produced other physiological changes ordinarily attributed to the corpus luteum, such as inhibition of ovulation, vacuolation of the vaginal mucosa of rats,[3] production of pseudo-pregnancy in rabbits,[4] and production of premenstrual endometrium in the uterus of castrate monkeys.[5]

The opinion that more than one hormone was present in our corpus luteum extracts was expressed in some of our earlier papers. This has proven to be the case, since we have obtained a crystalline fraction which produces relaxation of the pelvic ligaments of the guinea pig, while the remaining fraction contains the active material which is responsible for the other physiological reactions. This paper deals with the preparation of the crystalline substance together with some of its properties.

The minced lutein tissue is extracted, for 48 hours, with twice its volume of acid alcohol, at room temperature. The alcohol is acidified by adding 2 cc. of concentrated HCl to 98 cc. of 95% alco-

* Assisted by grants from the National Research Council, Committee on Problems of Sex.

[1] Hisaw, F. L., *J. Physiol. Zool.*, 1929, ii, 59.

[2] Hisaw, F. L., Fevold, H. L., and Meyer, R. K., *J. Physiol. Zool.*, 1930, iii, 135.

[3] Hisaw, F. L., Meyer, R. K., and Weichert, C. K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 754.

[4] Hisaw, F. L., and Leonard S. L., *Am. J. Physiol.*, 1930, xcii, 574.

[5] Hisaw, F. L., Meyer, R. K., and Fevold, H. L., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 400.

hol. The extract is removed and diluted with one-third its volume of water. The fatty material, which separates out, is filtered off and discarded. The extract is neutralized to a pH of 6.8 whereupon a voluminous precipitate settles out. This is filtered off, redissolved in acid alcohol and reprecipitated. The precipitate, after the second precipitation, is discarded and the second extract is added to the first. The united extracts are evaporated to semidryness in a fan oven at 37° C. and the residue is extracted with 95% alcohol. The alcoholic extract is evaporated to semidryness as before. The evaporated extract is emulsified in water and an equal volume of acetone is added to precipitate the phosphotides. These are filtered off and discarded, while the extract is again evaporated to semidryness and thoroughly extracted with acetone or ether to remove any remaining fats. The residue is then extracted with 97% alcohol, 200 cc. for every kilogram of material. Any insoluble material is discarded.

The 97% alcoholic extract is evaporated to semidryness as before and the residue is dissolved in glacial acetic acid. The solution is permitted to evaporate slowly at 35° C. until the acid has been removed. Crystals of a definite form appear. These are purified by dissolving away the brown syrupy material by means of 99% alcohol. The crystals are insoluble in the alcohol and by repeated extractions they are obtained in a pure state. They can be redissolved and recrystallized from glacial acetic acid. The crystals contain the relaxative hormone, while the alcoholic extract is entirely inactive with respect to the relaxation reaction.

The crystals, containing the hormone, are very characteristic in form and are identical, no matter from what source they are prepared. They are composed of sodium chloride and nitrogenous organic material in the proportion of 4 to 1. This proportion is very constant, as shown by analysis of crystals prepared at different times, both before and after recrystallization.

The product is soluble in water, forming a water clear solution, which when injected, produces the characteristic physiological reaction. Less than one milligram of crystalline material or two tenths of a milligram of organic material is sufficient to produce relaxation of the pelvic ligaments of a guinea pig in full oestrum. This represents approximately one gm. of fresh lutein tissue.

The relaxative hormone forms water soluble salts of hydrochloric, sulphuric and acetic acids, while with picric acid it forms a salt which is insoluble in water, alcohol and ether. By means of this insoluble salt we have purified the hormone to the point where 0.07 of a milligram will give a positive reaction in the relaxation reaction. The hormone is very labile, being destroyed by

heat, alkalies, and oxidation. On exposure to air in dry form, the activity gradually decreases. In aqueous solution at pH of 3.2 it is stable. Such solutions have been kept in the icebox for eight to twelve months with no detectable decrease in activity.

4878

Purification of Hormone of Corpus Luteum Responsible for Progestational Development and Other Reactions.*

H. L. FEVOLD, FREDERICK L. HISAW AND R. K. MEYER.

From the Department of Zoology, University of Wisconsin.

While the relaxative hormone of sows' corpora lutea produces no other physiological reaction, as far as we know, other than relaxation of the pelvic ligaments, a second hormone is responsible for such reactions as inhibition of ovulation, production of pseudopregnancy in rabbits, vacuolation of the vaginal mucosa of rats and production of a premenstrual endometrium in the uterus of monkeys. The physiologically active material, which is responsible for these reactions, is present in the fractions from which the relaxative hormone has been removed. We have, therefore, 2 separate and distinct hormones elaborated by the corpora lutea of the sow. The following reports the separation of the 2 hormones, and the preparation of a highly purified extract, containing the second hormone of the corpus luteum. For convenience we shall, in this paper, refer to this hormone as hormone "B".

The extract is prepared in exactly the same manner as that described in the previous paper,[1] for the relaxative hormone, up to the point where the active principles are taken up in 97% alcohol, with one important exception: Hormone "B" is somewhat soluble in acetone so ether must be used to remove the last traces of fatty material. The hormone is insoluble or very slightly soluble in ether, consequently the fats may be removed with no significant loss of hormone. The 97% alcoholic extract is evaporated to semidryness leaving a residue, which contains both of the corpus luteum hormones. From this point, either of two methods may be used to separate the hormones.

* Assisted by grants from the National Research Council, Committee on Problems of Sex.

[1] Fevold, H. L., Hisaw, Frederick L., and Meyer, R. K., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 604.

Procedure I. The residue, from the evaporation of the 97% alcoholic extract, is dissolved in glacial acetic acid and the relaxative hormone crystallized and purified as previously described. Hormone "B" is removed from the crystalline fraction by the extraction with 99% alcohol while the relaxative hormone remains insoluble in the crystalline form as previously described. The alcoholic solution is diluted with 99% alcohol until no more precipitate forms. The precipitate is thoroughly washed with alcohol and discarded, since it is inactive. The diluted alcoholic extract contains hormone "B" in purified form. The alcohol is removed by evaporation at 37° C., the residue extracted with ether and the ether insoluble material taken up in water in which it dissolves readily. The aqueous solution may be concentrated to any desired concentration with no turbidity appearing. The extract is sterilized by filtering through a Berkefeld or Seitz filter into sterile ampules which are then stored in the icebox.

Procedure II. The residue, after evaporating the 97% alcoholic extract, is thoroughly extracted with a large amount of 99% alcohol. The insoluble residue contains the relaxative hormone, while the alcoholic solution is entirely free from this substance but contains all of Hormone "B". This extract is then evaporated to dryness, extracted with ether, dissolved in water and prepared for injection as in Procedure I. The alcohol insoluble material is used as a source of the relaxative hormone.

Hormone "B" is soluble in alcohol and water and has more tendency to dissolve in fatty solvents than the relaxative hormone. It is more stable toward heat than is the relaxative hormone, also more stable toward oxidation, but is very sensitive toward alkali. In slightly acid solution it is quite stable. With picric acid it forms a dark brown oily product which is insoluble in water. The purification of this oily material is being carried out at the present time.

Hormone "B" may be extracted from fresh lutein tissue by means of neutral alcohol as described by Corner.[2] This leaves the relaxative hormone and it may be obtained by subsequent extraction with acid alcohol. However, the extract thus obtained is not as active for the relaxation reaction as an acid alcohol extract of the fresh tissue. We have, therefore, found it more convenient to obtain both hormones in solution by means of acidified alcohol and separating the two by the methods described.

The methods of fractionating the corpus luteum hormones as described here, with slight modifications, have also been used in the

[2] Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, lxxviii, 326.

separation of the gonad stimulating hormones of the anterior lobe of the hypophysis.[3]

The purified extract of hormone "B" is almost entirely free from the oestrus producing hormone. This is of great importance since it has been found that the action of hormone "B" depends to a large extent on the presence of the oestrus producing hormone. [4, 5] It is therefore desirable to have an extract free from oestrin in order to study the relationship of the 2 hormones in certain physiological reactions.

4879

A Renal Lesion Following Plasmapheresis.

M. HERBERT BARKER. (Introduced by A. C. Ivy.)

From the Department of Medicine, Northwestern University Medical School.

Chronic nephritis with edema (nephrosis) is associated with a marked albuminuria and a lowered total serum protein as pointed out by Epstein.[1] The importance of the level of the albumin fraction in relation to the appearance of the edema, both clinically and experimentally, has been emphasized by Barker and Kirk.[2] In an attempt to study the effect of the low proteinemia on the kidneys, a similarly low serum albumin level (about 1 gm. per 100 cc. of blood serum) has been produced and maintained in a series of dogs by plasmapheresis. Renal tissue has been obtained from these dogs by nephrectomy or by destroying the animal at periods varying from 2 weeks to 6 months. Gross and microscopic studies have shown the beginning and rather rapid progression of a degenerative renal lesion.

The first changes were noticeable at the end of 2 weeks at which time the kidney appeared swollen. The cortex was relatively thickened and was a brownish-gray color. Microscopically, cloudy swelling was noted particularly in the convoluted tubules together with desquamation of the tubular epithelium and extrusion of many nuclei. There was some hyaline droplet formation and occasional shrinkage of the glomerular tufts. After about one month of plas-

[3] Claus, P. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 29.

[4] Weichert, C. K., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 490.

[5] Hisaw, Frederick L., and Leonard, Samuel L., *Am. J. Physiol.*, 1930, xcii, 574.

[1] Epstein, A. A., *Am. J. Med. Sci.*, 1922, clxiii, 167.

[2] Barker, M. Herbert, Kirk, E. J., *New Eng. J. Med.*, 1929, 408.

mapheresis, the gross appearance of the kidneys was quite normal but microscopically, in addition to the cloudy swelling and breaking down of the tubular epithelium, there was fatty infiltration along the basement membrane of the tubules and there were small areas of round cell infiltration in the convoluted portions. An occasional glomerulus showed atrophy together with hyalinization and thickening of the capsule.

Tissue obtained at 2 months showed a still more marked atrophy of the tubules. Large areas of round cell infiltration and a definite connective tissue replacement was seen all through the inner half of the cortical tubular region. Numerous glomeruli showed an increased thickening of the capsule, increased hyalinization and atrophy. Four months showed further atrophy and scar tissue formation. At 6 months, the gross changes were pronounced. The capsule stripped easily but left a roughened and dimpled surface. The cortex was greatly narrowed and it appeared to be marked with grayish-white streaks. Microscopic examination revealed a marked scar tissue replacement in the inner half of the cortical tubular region with bands of scar tissue radiating to the surface producing the dimpling. There was a great increase in the tubular degeneration, fatty infiltration, round cell infiltration and glomerular atrophy over that seen earlier in the process. The blood urea nitrogen was not increased at any time in these animals.

These findings indicate that a secondary contracted kidney may well follow a long standing low proteinemia as a result of tubular atrophy and scar tissue replacement. It would also suggest an explanation of why most so-called nephrosis cases that escape intercurrent infections, die of uremia and at post-mortem show scarred and contracted kidneys.

4880

The Action of Irradiated Ergosterol on Rats and Chickens.

A. F. HESS AND G. C. SUPPLEE. (With the cooperation of O. D. Dow, G. E. Flanigan and O. J. Kahlenberg.)

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Research Laboratory, Dry Milk Co., Bainbridge, New York.

It is generally taken for granted that there is a parallelism between rickets, the percentage of ash in the bones, and the concentration of inorganic phosphorus in the blood, and furthermore that factors which prevent or cure rickets are associated with a tendency

to increase the bone ash and the phosphorus in the blood. In connection with infantile rickets, attention has been drawn from time to time by one of us to the fact that this disorder may be accompanied by a high concentration of inorganic phosphorus in the blood. In the course of an extended investigation, we have found that this same phenomenon may hold true for rats. Furthermore, a striking difference in this respect has been noted between the action of irradiated ergosterol and cod liver oil, when inadequate amounts of the former preparation are given. In a series of curative experiments it was found repeatedly that the inorganic phosphorus could be raised to normal concentrations, to 6 mg. or more per 100 cc. of serum, and that nevertheless no evidences of healing resulted, as judged by the "line test". Animals treated in this way had a low percentage of bone ash, about 30%, which is an amount indicative of rickets. These rats were about 4 weeks old, weighed approximately 50 gm. and had been fed the Steenbock rickets-producing ration plus 10 cc. of reconstituted dry milk. On the other hand, when cod liver oil was added to the diet, in amounts varying from 7 to 20 mg. daily, marked healing followed and the bone ash increased, but the concentration of inorganic phosphorus in the blood did not rise above 2 to 4 mg. per 100 cc. A result of this kind emphasizes the fact that the healing of rickets is not merely contingent upon a normal level of blood phosphate. It also shows the tendency of irradiated ergosterol to raise the phosphate concentration of the blood, quite apart from exciting any antirachitic or calcifying activity. When adequate amounts were added to the diet prompt and marked healing was brought about.

Recently chickens have been used by many in the study of rickets. For the past 3 years we have been carrying out experiments to ascertain the action of ultraviolet light, of irradiated ergosterol, and of cod liver oil on these animals. Without going into detail in regard to these experiments, we wish to point out in this connection two significant differences between chickens and rats in their reaction to specific antirachitic substances. In the first place it was found that whereas chickens are regularly protected against leg weakness by an addition of 1% of cod liver oil to their ration, a supplement of irradiated ergosterol equivalent to many times this amount failed to afford protection. It may be added that the ash of the bones was found to be comparatively low in the animals to which irradiated ergosterol had been given. This lack of response was all the more surprising, as chickens were found to respond readily to mild intensities of ultraviolet radiations and showed a high percentage of bone ash after an experimental period of 10 weeks.

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Irradiated Ergosterol. Maintenance of Blood Phosphate Level in the Course of Development of Rickets in Infants.

A. F. HESS, J. M. LEWIS AND H. RIVKIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In a previous communication[1] it was shown that rachitic rats may respond to small amounts of irradiated ergosterol by a marked rise of the inorganic phosphorus of the blood, unaccompanied by any signs of healing at the epiphyses. It has also been pointed out[2] by us that this phenomenon held true for infants who received dry milk which had been inadequately irradiated. In this communication we wish to add the observation that some infants which had been getting irradiated ergosterol (viosterol) for a period of several months, in the course of the winter, reacted in a similar way. These infants had been given small amounts of a standard preparation of irradiated ergosterol. Evidently in these instances this quantity was insufficient to afford complete protection; had a more potent preparation been given, complete protection would have resulted.

Irradiated foods and sterols have a tendency to raise the phosphorus in the blood, irrespective of and apart from their antirachitic action. Therefore, an analysis of the blood does not give reliable information as to the progress of the rachitic condition. Under these conditions, information in regard to the presence of rickets can best be obtained by means of direct clinical examination or roentgenologic pictures.

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[1] Hess, A. F., and Supplee, G. C., PROC. SOC. EXP. BIOL. AND MED., 1930, xxvii, 608.

[2] Hess, A. F., Lewis, J. M., Rivkin, H., *J. Am. Med. Assn.*, 1929, xciii, 661.